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PURPLE SEED STAIN OF SOYBEAN[1]

HARRY H. MURAKISHI[2]

(Accepted for publication August 1, 1950)

A disease of soybean, *Glycine max* (L.) Merrill, recognized chiefly by means of a purple discoloration of the seeds and frequently called purple seed stain, purple spot, purple blotch, and lavender spot, has been reported from several localities in the United States since 1926 (6, 7, 8, 12, 15, 16, 21). The discoloration is objectionable to seed producers because it means difficulty in securing seed certification (7, 8).

The first report of a disease causing purple stain of soybean seeds was made in the Orient in 1921 by Suzuki (28), who attributed the cause to climatic factors. Later, Matsumoto and Tomoyasu (18) demonstrated the causal agent to be an undescribed fungus which they named *Cercosporina kikuchii* after R. Kikuchi who, according to these workers, found the fungus on diseased seeds in 1922. Symptoms on seeds, seedlings, and foliage of older plants were described by Matsumoto and Tomoyasu for the disease occurring in Japan. In the Orient, the disease has been called purple speck and purple patch.

In the United States, Gardner (6) briefly noted a similar disease of soybean seeds in 1924 and attributed the discoloration to the presence of the mycelium of a Cercospora in the seed coat (7). However, up to the present, no report has been published on the morphology, cultural features, and pathogenicity of the fungus associated with purple stain in the United States. The present paper, in addition to an account of these phases, describes the symptoms and the seasonal development of the disease and gives results of preliminary seed-treatment experiments in the control of the disease.

To date, the disease has been reported only on soybeans. It has been observed in North Carolina on seeds of the following field varieties: Herman, Haberlandt, Ogden, Rose Non Pop, Wood's Yellow, Volstate, Arksoy, Tokio, S–100, and Roanoke. It is also known to occur on other field types such as Boone, Manchu, Yokotan, Biloxi, Morse, Hoosier, and Habaro; and on the vegetable types Easy Cook, Lima Soy, and Rokusun. It has also been reported on the varieties Dunfield, Midwest, Mansoy, and Ito San in Indiana (8).

[1] A revision of a section of a thesis submitted to the Graduate School of the University of North Carolina at Raleigh, in partial fulfillment of the requirements for the degree of Master of Science.

Contribution from the Plant Pathology Section, Department of Botany, North Carolina Agricultural Experiment Station. Published with the approval of the Director as Paper No. 342 of the Journal Series.

The author is indebted to Dr. Samuel G. Lehman, under whose direction this work was completed, and to other members of the Plant Pathology Section for suggestions in the preparation of the manuscript.

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SYMPTOMS

On seeds, the disease is characterized by irregular blotches varying from light to dark purple and ranging from a tiny spot to the entire area of the seed coat. These discolorations are often accompanied by wide cracks in the seed coat usually extending transversely along the seed (Fig. 1). The

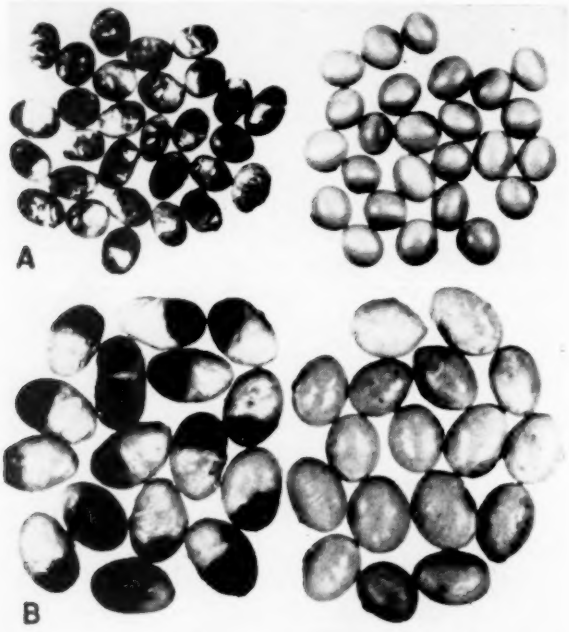

FIG. 1. At left, purple stain on seeds of S–100 (A) and Rokusun (B); natural infection. Healthy seeds on right.

purple is visible on the inside of the seed coat, but cotyledons are generally not discolored even on severely diseased seeds. When diseased seeds germinate, the cotyledons often become shrivelled and dark purple. Cotyledonary infection spreads to stems of young seedlings, producing a dark, diseased area which may later encircle the stem. Usually plants thus affected are killed. Those less severely attacked survive but are stunted and appear weaker than healthy plants. Within 1 to 2 weeks after germination, a velvety, grayish-white growth of conidia and conidiophores of the causal fungus occurs on the diseased portion of cotyledons and stems. Infected primary and secondary leaves have reddish-purple, angular to irregular spots on both upper and lower leaf surfaces. These vary from a pin-point spot to irregular patches up to 1 cm. in diameter (Fig. 2, A). There is no perceptible chlorosis at the margin of the reddish-purple diseased tissue. When infections are numerous, however, the leaves become yellow prematurely. Young infections on stems and petioles of older plants are characterized by slightly sunken, irregular, reddish-purple areas, one to several millimeters in length (Fig. 2, B). Several infected areas may coalesce so that the stem

or petiole becomes completely encircled. The disease at this stage has not been observed to kill the plant, although severe infection of petioles may cause premature falling of leaves. Areas recently infected on maturing pods are minute, reddish to reddish-purple, but later become purplish-black.

THE CAUSAL FUNGUS

Isolation. The causal fungus was readily isolated in pure culture. When surface-sterilized seeds were planted on dextrose agar, the fungus

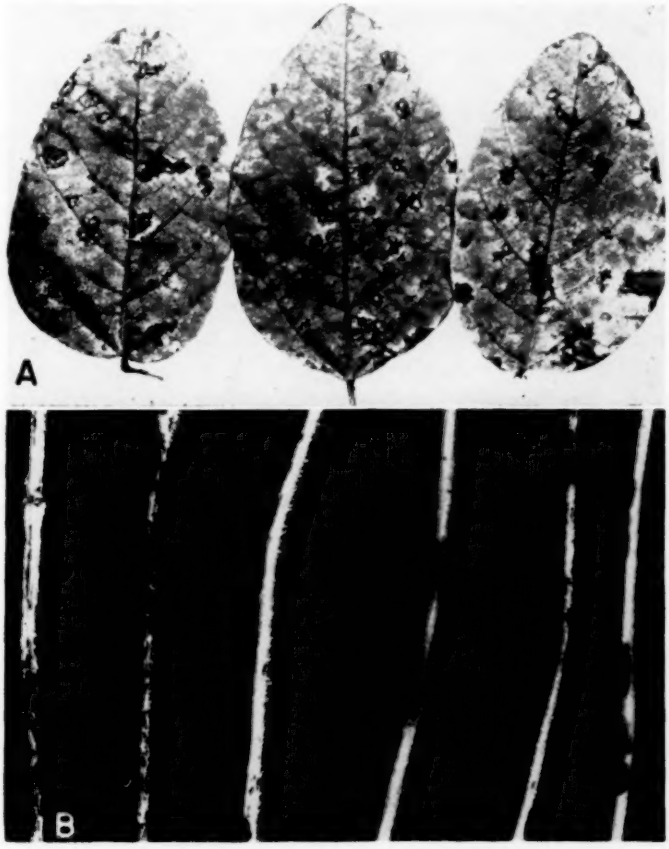

FIG. 2. Symptoms produced on foliage of S–100 plants following greenhouse inoculations with *C. kikuchii;* advanced lesions on leaves (A) and stems (B).

mycelium grew out in 1 week, coloring the medium a characteristic purple. Suspensions of conidia washed from lesions on stems, leaves, petioles, or cotyledons and streaked with a wire loop across a hardened plate of dextrose agar gave rise to discrete colonies with typical mycelial growth and purple coloration of the medium. A more satisfactory method was to place an infected leaf, stem, petiole, or seed in a moist chamber for 48 to 72 hr. at 23° to 27° C., after which abundant conidia were produced. By use of a dis-

secting microscope, the conidia were picked off with a moistened transfer needle and planted directly on dextrose agar.

Identity and description. In agar culture media, the young hyphae of the purple-stain fungus are hyaline, slender, 2–4 μ in width, with granular contents, septate, sometimes noduled. Later the hyphae become thicker, 3–5 μ in width, more closely septate, and pale to medium brown. In old cultures orange-brown, thick-walled cells resembling chlamydospores are formed. These large cells vary from 6 to 15 μ across and are formed in chains.

The fungus has thus far failed to sporulate in culture. For that reason, all observations on the morphological details of the conidiophores and conidia have been made from fruiting bodies found on diseased plant parts. Conidiophores develop profusely on diseased seeds kept for 36 hr. in moist chambers at 23° to 27° C. These also occur on diseased hypocotyls and cotyledons of seedlings and on stems, petioles, and leaves of older plants. Fruiting occurs on both the upper and lower leaf surfaces, but is more abundant on the lower surface. The conidiophores are pale to medium brown and sparingly septate. They may occur singly but usually are in fascicles of from three to as many as 20 or more. The conidiophores arise from a dense cluster of a few to many irregular to round, brown cells which may be called a stroma. On germinating seeds the stroma develops within the seed coat. On leaves the stroma develops just below the epidermis, the fascicles of conidiophores arising directly from the basal stroma (Fig. 3, C). On stems and petioles, however, the stroma is deep within the cortex. From this basal stroma arise loosely-arranged chains of hyphal cells (Fig. 3, D). A single conidiophore is formed at the end of each hyphal chain near the epidermis. A fascicle of conidiophores is produced by a group of chains of hyphal cells. The conidiophores average (100 measured) 157 μ long and 4–6 μ wide, are brownish with hyaline tips, and have conspicuous spore scars. They become geniculate with age, often having one to two kneelike joints. The conidia are slender, straight to variously curved, acute at the tip, truncate to subtruncate at the base, and average 210 μ long and 3.3 μ wide. Septations vary from 2 to 49 in number (Fig. 3, A). Conidia germinate readily on dextrose agar within 3 to 4 hr. Germ tubes emerge from cells at either or both ends of the conidia and often from other cells as well (Fig. 3, B).

Measurements of conidia and conidiophores found on leaves and stems of the soybean variety S-100 grown in the greenhouse in Raleigh, North Carolina, were compared with those reported by Matsumoto and Tomoyasu (18) in the Orient. The fungus studied in North Carolina had greater range in length, width, and septation of conidia and in length and width of the conidiophores. Conidiophores found on leaves in Japan (18) were 85–220 $\mu \times 4$–6 μ; conidia were 70–165 $\mu \times 4$–5 μ; number of septa, 0–22. Conidiophores taken from leaves and stems at Raleigh measured 36–296 $\mu \times 3.2$–6.1 μ, and conidia were 38.8–445 $\mu \times 1.3$–6.1 μ; conidial septa, 2–49.

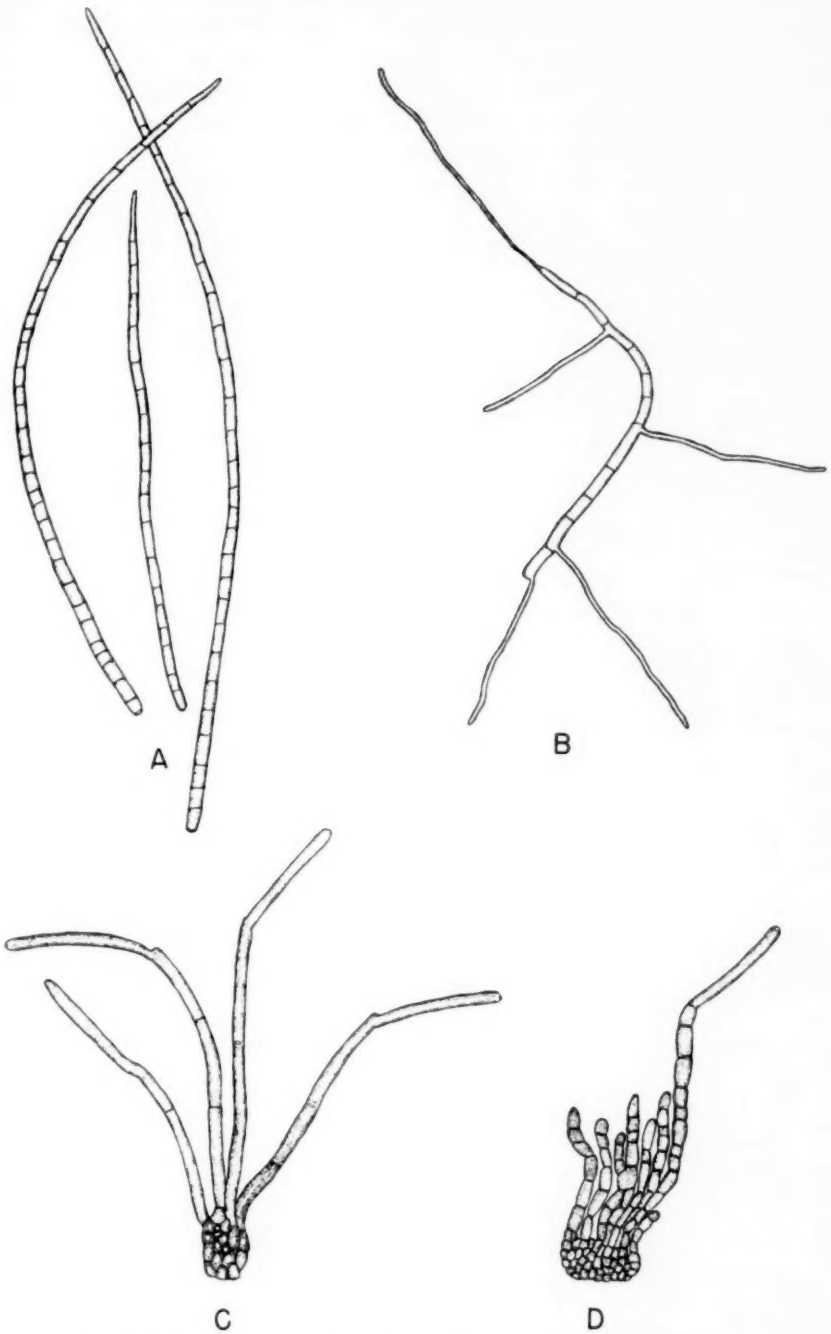

FIG. 3. Fruiting structures of *C. kikuchii* produced on greenhouse-inoculated plants (camera-lucida drawings × 400). A. Variation in size and form of conidia. B. Germinating conidium after 10 hr. on dextrose agar. C. Fasciele of conidiophores of a type found on diseased leaves. D. Single conidiophore developing from a chain of hyphal cells; this type of fruiting structure found on diseased stems and petioles.

The length and the number of septations, especially of the conidia, were found to be greatly influenced by humidity. On diseased stems kept in saturated atmosphere, length of 50 conidia varied from 123 to 445 μ and number of septations from 12 to 49; while a like number of conidia on diseased stems in the greenhouse (average relative humidity 60–65 per cent) ranged from 38.8 to 300 μ in length and 6 to 37 in number of septations. These observations on the effect of humidity on length of conidia are in agreement with those of other workers with fungi (11, 30). The wide range in conidia and conidiophore measurements obtained by the writer may be due to the different levels of humidity under which the North Carolina fungus was allowed to sporulate. The conidia and conidiophore measurements given by Matsumoto and Tomoyasu fall within the range found for the respective measurements of the fungus studied in North Carolina. Furthermore, the shape, color, and septation of the fruiting bodies of the isolates in question are essentially the same, so far as can be determined from published descriptions. Therefore, the purple-stain fungus herein described is considered to be the same as *Cercosporina kikuchii* described by Matsumoto and Tomoyasu.

CULTURAL CHARACTERISTICS

Cultural characters have been proposed as aids to the identification of species of fungi by various workers (3, 30). Matsumoto and Tomoyasu (18) noted the production of purple color by *C. kikuchii* in media containing dextrose. The North Carolina isolates of *C. kikuchii* were grown on various culture media as an aid to identification and as a possible means of inducing sporulation. Standard laboratory formulae (25) were used for all media except soybean plant extract agar. For this medium the extracted juice from young soybean seedlings was first passed through a Seitz filter; then added to poured plates of dextrose agar when the agar had cooled to just above the solidifying point. Petri-dish cultures of all media were made in triplicate and held at room temperature (20°–25° C.) for 10 days, after which observations on cultural characteristics were recorded. Color determinations were based on Ridgway's Color Standards and Color Nomenclature (24). Fifteen kinds of media were used; a description of the growth on six kinds is given below.

Potato-dextrose agar.—Aerial growth abundant, uniformly dense, deep folds radiating from the center, white on edges to light grayish-olive toward center; substratum beneath the colony dark maroon-purple with cameo-pink margins; Indian Lake pigment diffuses into the medium. The best mycelial growth is made on this medium.

Dextrose agar.—Aerial growth thin, sparse, purplish lilac at margin, citrine drab at center; concentric rings of growth present; substratum purplish lilac at edges, deep olive at center; purplish-lilac pigment diffuses into the medium.

Dextrose agar with sterile plant extract.—Aerial growth very abundant,

mineral-gray at margin grading into olivaceous black at center; droplets of green liquid in center region; substratum dusky olive green to yellowish glaucous at margin.

Soybean-seed agar.—Aerial mycelium abundant, uniformly dense, deep radial folds, white to dark, dull, blackish green; substratum anthracene purple with light vinaceous-purple margin.

Soybean-leaf agar.—Aerial mycelium abundant, uniformly dense, deep radial folds, American green at center, dull blackish green grading into greenish glaucous at the edges.

Czapek's medium with 2 per cent agar added.—Aerial mycelium dense, white; substratum dull dusky purple to dull dark purple at margin; Indian Lake pigment diffuses into medium around colony.

Spore Production and Growth

No conidia were found in cultures on any of the media described above. It has also been the experience of others (7, 12) that isolates of this fungus in America have consistently failed to produce conidia in pure culture. Similar difficulties have been reported for other species of fungi (5, 22, 23, 27, 29, 31). In order to determine the factors necessary for spore formation of *Cercosporina kikuchii* in pure culture, a study was made of the effect of various conditions and factors found by previous workers to be favorable for spore production in *Cercospora*, a genus to which *Cercosporina* is closely allied.

Effect of temperature. Matsumoto (17) found the optimum temperature for spore formation of *C. kikuchii* on diseased seeds to be between 15° and 25° C. The present writer observed spore formation within 2 to 3 days on diseased stems, leaves, petioles, and seeds kept in moist chambers at room temperature (20°–25° C.). In order to obtain information regarding the temperature range in which *C. kikuchii* produces conidia, controlled tests were made. Diseased seeds were surface sterilized in 1 : 1000 mercuric chloride for 2 min., washed in several changes of sterile water, and placed in Petri dishes lined at the bottom with moistened filter paper. Duplicate sets of dishes, each containing 20 purple-stained seeds, were held at constant temperatures of 10°, 16°, 18°, 21°, 23°, and 27° C. Microscopic examinations made at 12- to 14-hr. intervals revealed that conidia were produced after 48 to 72 hr. of incubation on seeds held at 18°, 21°, 23°, and 27° C. The optimum for spore production on diseased seeds was between 23° and 27° C. At 18° and 21° C. sporulation was sparse; no conidia were produced on seeds held at 10° and 16° C.

Only Matsumoto (17) and Matsumoto and Tomoyasu (18) have reported the occurrence of conidia of *C. kikuchii* in pure culture. They observed conidia in cultures held at 25° C. on raisin agar (18) and also on potato-dextrose agar (17). Nagel (20) found temperatures between 23° and 27° C. were favorable for spore formation of *Cercospora beticola* and several other species of *Cercospora;* Schmidt (26) obtained sporulation in *Cerco-*

spora beticola by subjecting cultures of the fungus to alternating temperatures.

In order to test the effect of different constant and alternating temperatures on growth and sporulation, two series of subcultures of *C. kikuchii* were made in triplicate on potato-dextrose agar. The first was held at 12°, 15°, 18°, 22°, 24°, 27°, 30°, 35°, and 40° C. The fungus grew very little at 12° C. and failed to grow at 40° C. Optimum temperature for growth was 27° C. The second series was subjected to daily alternating temperature: 24° C. for 24 hr., after which the cultures were placed at 18° C. for 24 hr. and then back to 24° C., and so on. No spores were produced at any temperature during a 1-month period of treatment and observation.

Effect of pH. To test the effect of the hydrogen-ion concentration on conidial production and mycelial development, the following medium was used: KH_2PO_4, 1.0 gm.; $MgSO_4$, 0.5 gm.; peptone, 5.0 gm.; agar, 17.0 gm.; distilled H_2O, 1000 ml. The medium was adjusted to the desired pH by the addition of sterile potassium hydroxide or tartaric acid just before the plates were poured. Growth occurred at a pH range of 3.9 to 8.6; optimum pH for mycelial development of *C. kikuchii* was 5.9. No spores were produced in any of the plates.

Other methods reported by different workers to be effective means of inducing sporulation were tried with the soybean purple-stain fungus. Maceration of the mycelium (9), propagation of cultures by conidial transfer (19), variation of moisture content of the substrate (10, 13, 19), cultivation on autoclaved host tissue (18), and growth on host decoction media (2, 4, 14, 19) failed to induce conidial production in cultures of the purple-stain fungus held at several temperature levels. No conidia were found in numerous cultures exposed to sunlight for varying periods of time.

PROPERTIES AND NATURE OF THE PIGMENT CAUSING PURPLE COLOR OF MEDIUM

Effect of pH on pigment. Matsumoto and Tomoyasu (18) noted that the substance causing purple color produced by *C. kikuchii* could be extracted with alcohol; it was red in acidified alcohol and green in alkaline alcohol, but the pH range at which the purple color is produced was not stated. Tests have been made by the writer to determine this pH range.

Cultures of the fungus were grown on potato-dextrose agar for 2 weeks, after which the mycelium and the discolored portions of the medium from 12 Petri-dish cultures were stored overnight in alcohol acidified with hydrochloric acid. The acidified alcohol containing the extracted substance was then filtered free of mycelium fragments and bits of agar. The pH of the acidified alcoholic extract after filtration was 2.1. Fifth normal solutions of hydrochloric acid and potassium hydroxide were used in titrating the alcoholic extract and pH measurements were determined electrometrically.

At pH 2.1 to 6.1 the color of the extract was coral red to vinaceous rufus (Ridgway, Color Standards and Color Nomenclature, 1912); at pH 6.2, dark vinaceous; at pH 6.4 to 8.5, dark vinaceous rufus; at pH 9.1 to 9.4,

suntan brown to amber-brown; at pH 9.5 to 9.6, olive citrine to citrine drab; at pH 9.7 to 10.8, ivy green; and at pH 10.9 to 12.0, parrot green.

Definite purple color was produced between pH 6.4 and 8.5. As shown previously by Matsumoto and Tomoyasu, red was produced in the extreme acid range and green in the extreme alkaline range. Change of color during growth of *C. kikuchii* appears to be related to the effect of hydrogen-ion changes on the pigment produced by the fungus.

Solubility of pigment. Five-cc. portions of the acidified alcoholic extract were mixed with a number of solvents and allowed to stand for 30 min. The solubility of the pigment in the respective solvents was judged by whether the red pigment appeared in the chemical added, or remained in the alcohol. The pigment was found to be soluble in ether, chloroform and acetone, slightly soluble in turpentine oil, and insoluble in carbon bisulfide, benzin, and water. At first it appeared insoluble in benzene but dissolved in this chemical after 30 min. The results of this test indicate similar solubility characteristics for the pigment secreted by the fungus isolates studied by Matsumoto and Tomoyasu and by the writer.

PATHOGENICITY STUDIES

In 1944 as high as 32 per cent of the seeds from the variety S-100, grown at Plymouth, North Carolina, had purple discoloration. The discolored areas on the seeds ranged from tiny spots to large patches, often entirely covering the seed.

One hundred purple and 100 healthy-appearing seeds were selected from North Carolina-grown varieties S-100 grown at Plymouth and S-100 and Rokusun grown at McCullers. The seeds were surface-sterilized in 1 : 1000 mercuric chloride after a 10-sec. dip in 70 per cent ethyl alcohol, washed in several changes of sterile water, and then planted on dextrose agar in test tubes. After 2 weeks at laboratory temperature, observations were made to determine the percentage of germination and the kind of organisms found on the purple and healthy seeds. *C. kikuchii* was present in 87–91 per cent of the purple seeds planted and in 4–10 per cent of the seeds which appeared healthy (Table 1). The healthy lot had many more sterile seeds than did the purple lot. The germination counts on the agar slants for the purple seeds were from 12 to 25 per cent lower than those for the healthy seeds.

Pure cultures of *C. kikuchii* isolated from diseased hypocotyls and grown on potato-dextrose agar plates for 2 weeks were reduced to a fine mycelial suspension in a mechanical liquefier, according to a method described by Andrus (1). Sixty-five healthy, 5-week-old, S-100 plants growing in flats of steamed sand in the greenhouse were atomized with the mycelial suspension. Eighty-four plants of the same variety and the same age were treated identically except that no inoculum was used. The plants were kept in a moist compartment for 10 days, after which they were removed to greenhouse benches. Typical reddish-purple lesions began to appear on stems and petioles the tenth day after inoculation. Spots on leaves were visible

TABLE 1.—*Percentage germination and kind of organisms found on three samples of soybean seeds grown in North Carolina in 1944*

Variety and locality	Condition of seed	Percentage germination on agar	Organism associated, in percentage of seeds plated			
			Sterile	*C. kikuchii*	Bacteria	Miscellaneous fungus[a]
S–100	Purple	62	1	91	0	8
(Plymouth)	Healthy[b]	83	83	10	1	6
S–100	Purple	64	0	87	6	7
(McCullers)	Healthy	89	91	4	2	3
Rokusun	Purple	13	2	91	5	2
(McCullers)	Healthy	25	33	5	34	28

[a] Miscellaneous fungi included Alternaria, Colletotrichum, Mucor, and Fusarium spp.
[b] Apparently healthy.

after 14 days from the time the mycelial suspension was applied. Sixty-one of the 64 inoculated plants became infected; the checks remained free of disease.

Pod inoculations were made in the laboratory and greenhouse. For the laboratory inoculation, nearly mature field-grown pods of the Herman variety were surface sterilized in alcoholic mercuric chloride solution for 2 min., washed in sterile water, placed in Petri dishes, and kept at room temperature. Twenty-five pods were wounded with a sterile scalpel and inoculum consisting of a mycelial culture obtained from a purple seed was placed on the outside of the pod walls. Inoculum was placed similarly on 20 unwounded pods (Table 2). Greater infection resulted when pods were wounded; however, infection did take place on unwounded pods.

TABLE 2.—*Infection of detached soybean pods inoculated with C. kikuchii in the laboratory*

Treatment	Number of seeds		Total	Percentage of seeds infected
	Purple	Not purple		
Not wounded; inoculated	28	13	41	68.3
Wounded; inoculated	36	3	39	92.3
Control, not wounded; no inoculum	3	36	39	7.7

The fungus was reisolated from the inoculated seeds and produced purple coloration of dextrose agar. Purple seeds found in the control are believed due to natural infections which occurred while the pods were maturing on field-grown plants.

Inoculations with a mycelial culture were also made on pods on plants of the S-100 variety growing in flats of steamed sand in the greenhouse. After treatment and inoculation (Table 3), the pods were individually wrapped in waxed paper and the plants were kept in a large moist compartment in the greenhouse for 7 days, then removed to the bench. Eleven days after inoculation, the plants were examined to determine the number of seeds infected.

TABLE 3.—*Infection of soybean pods following inoculation in the greenhouse with (A) a mycelial culture and (B) conidia of C. kikuchii*

Treatment	Number inoculated		Number of seeds		Percentage of seeds infected
	Plants	Pods	Purple	Not purple	
A					
Not wounded; inoculated	34	57	0	111	0.0
Wounded; inoculated	35	49	83	12	87.4
Control, not wounded; sterile agar	26	42	1	93	1.1[a]
Control, wounded; sterile agar	27	40	0	88	0.0
B					
Not wounded; inoculated	17	32	0	72	0.0
Pods punctured; inoculated	20	26	20	40	33.3
Control, wounded; atomized with distilled water	12	25	0	57	0.0

[a] Believed due to natural infection.

A high percentage of infection occurred when wounded pods were inoculated (Table 3), but contrary to results in the laboratory experiments, no infection resulted on unwounded, inoculated pods. In the laboratory, conditions believed to be ideal for infection prevailed in the Petri dishes which were kept moist with damp paper (Table 2). It seems that very high relative humidity is necessary for seed infection when the pod wall is unbroken.

Inoculated leaves, stems, and petioles of the greenhouse-test plants which had reddish-purple spots symptomatic of the disease were surface-sterilized, washed in sterile water, and kept in moist chambers for 3 days at room temperature. Abundant spores were produced. These spores were planted on dextrose agar and in 4 to 5 days typical mycelial growth occurred and the characteristic purple in the medium was produced. Using conidia washed from the inoculated leaves, stems, and petioles mentioned above, further inoculations were made by atomizing the conidial suspension onto pods of S-100 plants in the greenhouse. No purple seeds occurred in unwounded inoculated pods, whereas 33.3 per cent of the seeds in wounded pods became purple (Table 3).

The results of the three tests, summarized in tables 2 and 3, indicate that infection may occur on unwounded, detached pods under conditions of high humidity, and that wounds in the pod wall greatly increase the chances of infection. The experiments indicate the further possibility that pods attached to growing plants may become infected only through wounds.

DEVELOPMENT OF THE DISEASE IN THE GREENHOUSE

On diseased seeds the mycelium is confined chiefly to the seed coat. When seed coats and cotyledons from a number of purple-stained seeds were plated out separately on dextrose agar, typical mycelial growth and the

purple coloration of the agar characteristic of *C. kikuchii* were produced from all of the seed coats plated; cotyledons, however, rarely yielded the fungus.

To study natural infection and development, purple seeds of Arksoy, S-100, and Rose Non Pop varieties were grown in sterilized, potted soil in an enclosed cage covered with muslin sheets on all sides. The relative humidity was kept at a high level by a continuous trickle of water applied to the side of the cage from a canvas hose. When the purple seeds emerged from the moist soil, it was observed that the infections spread from seed coats to cotyledons and then to hypocotyls. Conidia were found on these infected areas. Ten days after these conidia had formed on cotyledonary leaves, purple infections were noticed on primary leaves. Infections continued to increase on primary leaves and soon new infections appeared on secondary leaves and on stems and petioles. New crops of conidia were found on all these infected parts. Since the plants remained in a vegetative state and produced only abortive seed pods, observations were not carried beyond this stage. It is assumed that conidia produced on infected parts of the foliage finally infect maturing pods and seeds, as demonstrated in the pathogenicity tests.

LABORATORY STUDIES ON CONTROL

Laboratory studies were made to determine whether purple stain could be controlled by certain seed treatments. Small samples of purple seeds were treated as follows: 1) 4 per cent formaldehyde dip, 2) hot water (44° C.) for 1 hr., 3) hot water (48° C.) for 30 min., and 4) hot water (48° C.) for 1 hr. Treatments 2 and 4 showed the most promise for disease control.

In a second test 100 purple and 100 healthy seeds were placed in separate hot-water baths at 48° C. for 1 hr. A third lot of 100 purple seeds was placed in a hot-water bath at 44° C. for 1 hr. For a control, 100 purple-stained seeds from the same source were left untreated. After the hot-water treatment, all seeds including the control were surface-sterilized in $HgCl_2$ 1:1000 for 2 min., washed in sterile water, and planted on dextrose agar slants. After 2 weeks, examinations were made to detect the presence of *C. kikuchii* and other organisms.

TABLE 4.—*Effect of hot-water treatment on percentage germination and on survival of organisms in seeds*

Temperature and duration of treatment	Condition of seed	Percentage of germination after treatment	Organisms in seeds after treatment[a]		
			Sterile	*C. kikuchii*	Misc'l organisms[b]
44° C./1 hr.	Purple	77	81	11	8
48° C./1 hr.	Purple	70	93	2	5
48° C./1 hr.	Healthy	59	93	0	7
Control	Purple	90	26	63	11

[a] As per cent of seeds planted.
[b] Included bacteria, Mucor spp.

The purple-stain fungus grew from 63 per cent of the control seeds (Table 4), but only 11 and 2 per cent of the purple seeds given the hot-water treatment for 1 hr. at 44° and 48° C., respectively, yielded *C. kikuchii*. The purple seeds of the control gave a higher germination percentage than did the seeds of the hot-water treatments.

SUMMARY

The fungus commonly associated with the purple-stain disease of soybean seed in North Carolina has been demonstrated to be the causal organism. Symptoms on hypocotyls, stems, leaves, and petioles, hitherto unreported in America, and on seeds and pods are like those reported in the Orient.

The morphological details and the cultural characteristics of the fungus found in purple seeds in America and in the Orient are closely similar. The results of these studies lead the writer to believe that the causal organism of purple stain in the United States and *Cercosporina kikuchii* Mats. & Tomo., the cause of a similar disease in the Orient, are identical species.

The conidial stage of *C. kikuchii*, heretofore not reported in the United States, is described in some detail and an account of the development of the disease is given.

The fungus sporulates on living seeds in the laboratory at temperatures of 18° to 27° C. It grew well but failed to sporulate on any one of a wide variety of laboratory culture media.

Laboratory tests showed that the purple-seed-stain fungus in a high percentage of infected seeds can be inactivated by immersing the seeds for 1 hr. in water at 48° C. This treatment caused considerable reduction in germination of seeds.

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FACTORS INFLUENCING THE INFECTION OF TABLE GRAPES BY BOTRYTIS CINEREA (PERS.)[1]

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(Accepted for publication November 1, 1950)

Botrytis rot of grapes, often called gray rot, gray mold, bunch rot, or slipskin, has been for many years a serious problem in the vineyards, in cold storage, in transit, and in the market. In California, especially in years when grapes were still on the vines at the time of the first fall rains, growers of Emperor and Tokay table grapes have sustained heavy losses. Harvesting is usually suspended for 3 to 5 days in the event of rain. By then, incipient infections have usually developed, so that symptoms are evident and infected berries can be removed from the bunches before they are packed.

Efforts to develop more satisfactory control measures for botrytis rot of grapes, both in the vineyard and in storage, have been hampered by the variability in seasonal development of the disease and by failure to obtain consistent infection of healthy grapes under a given set of conditions.

FACTORS INFLUENCING INFECTION

Infection courts. Vanderwalle (10) was able to infect injured, but not uninjured, grapes with conidia of *Botrytis cinerea.* Du Plessis (4) found wounded grapes susceptible to infection, even when half grown. Müller-Thurgau (8) concluded that *B. cinerea* readily infected either mature or immature injured grapes. Guillon (5) and Istvanffi (7) reported spread of the organism from diseased to healthy berries by contact infection.

In the present investigation, it was noted that many early lesions on grape berries were so located that contact infection was improbable, and no wound could be discerned. To investigate the possibility of insect punctures as possible courts of infection (5, 10), 600 mature berries of the variety Tokay with no visible injuries were placed in a 0.1 per cent Trypan blue solution for 16 hr. and then rinsed with distilled water; injuries showed distinctly as small spots where the dye had diffused into the subepidermal tissue and were so distributed as to indicate probable insect-puncture origin. The berries, arranged one layer deep in shallow baskets, half of them with an injured area and half with an uninjured area uppermost, were inoculated with a spore suspension of *B. cinerea* applied with an atomizer; they were kept wet with a fine intermittent spray of tap water for 48 hr., and then dried. Three days later, early lesions were detected by

[1] A portion of a thesis submitted to the graduate division of the University of California at Berkeley, in partial satisfaction of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his appreciation to Dr. William B. Hewitt and other members of the Division of Plant Pathology for counsel and advice in this work.

slight pressure on the berries which caused the weakened skin to crack over the infected area. Although accurate counts could not be made of the actual number of lesions with respect to injured and noninjured areas, it was evident that some lesions coincided with the site of the stained injured areas.

Müller-Thurgau (8) stated that infection of grapes by *B. cinerea* was often brought about by direct penetration through lenticels. In the present investigation, 405 mature Tokay berries were treated with the Trypan blue dye solution as before. After they were rinsed and dried, 202 berries were dipped quickly into a paraffin bath kept at about 70° C., so that the berry was nearly covered with a thin layer of paraffin. On each berry, only one lenticel was left exposed near the center of an area about 3 mm. in diameter. Another group of 203 berries was similarly dipped, except that the exposed areas contained no lenticels. No injuries, as indicated by the dye, were left exposed on any of the berries in either group. The exposed parts of the berries were inoculated and kept moist as before. In the group with lenticels exposed, 49 per cent of the berries were infected, and in that with no lenticels exposed, 52 per cent. Infection appeared to occur irrespective of the presence of the lenticels.

Moisture. Severe outbreaks of botrytis rot in the vineyard are usually preceded by rain. Guillon (5) showed that the spread of gray mold was closely associated with rainfall; rapid development followed periods of heavy rain. Rattray (9) reported that outbreaks of the disease in South Africa usually occurred within 3 days after a rain. Du Plessis (2, pp. 48–52) observed that the amount of wastage of grapes during storage, particularly that due to *B. cinerea*, was related to the amount of rainfall and high relative humidity immediately before and during the time of harvesting and packing. In another paper (1), du Plessis stated that *B. cinerea* required 33 hr. of moisture at 25° C. to infect grapes.

In an experiment designed to represent a period of rain, 70 clusters of mature Tokay grapes, averaging about 1.5 lb. each, were gleaned of injured berries and suspended by strings in 55-gal. cans serving as moist chambers; the clusters were kept wet, but without run-off, by a very fine intermittent spray of tap water. Clusters were inoculated by atomizing them with a tap-water suspension of botrytis conidia from diseased fruit; they were kept wet for 2 days, then allowed to dry. As a control, 70 clusters inoculated with conidia blown from diseased fruit were suspended in dry cans covered with lids adjusted to allow free ventilation.

Ten clusters chosen at random were withdrawn from each of the wet and dry chambers at 1-day intervals from 3 to 7 days and examined immediately. The percentages of infected berries for each lot of 10 clusters were: in the wet lots, 3 days, 68.7; 4 days, 72.0; 5 days, 78.3; 6 days, 86.2; and 7 days, 96.2; in the dry lots, 3 days, 2.0; 4 days, 2.9; 5 days, 4.7; 6 days, 4.3; and 7 days, 7.3. The presence of free water favored the development of botrytis infection.

Temperature. The foregoing experiment was slightly modified and re-

peated three times, to study the effect of the diurnal range of temperature on the length of the moisture period necessary to produce infection. Ninety clusters of grapes were used in each test; 80 were inoculated with a spore suspension in tap water and kept wet, and 10 were inoculated with dry conidia and kept dry. The temperature inside of the chamber was recorded by means of an air-soil thermograph placed near the chambers with the soil tube in one of them.

Ten clusters were withdrawn from the wet chambers at 6-hr. intervals from 12 to 48 hr.; another lot after 60 hr., and the last lot after 72 hr. Each lot was dried in an air stream for 6 hr., then placed in a dry chamber for incubation in the area adjacent to the original chambers. On the sixth day after inoculation, the fruit was examined and the percentage of infected berries determined.

Trial 1, with Tokay grapes, was made in a room whose temperature varied from 12° to 21° C., average 16° C. Trial 2, also with Tokay grapes, was made outside in the shade of a building, the temperature varying from 2° to 19° C., average 12° C. For trial 3, with Emperor grapes in the outside chambers, the temperature ranged from 2° to 21° C., average 12° C.

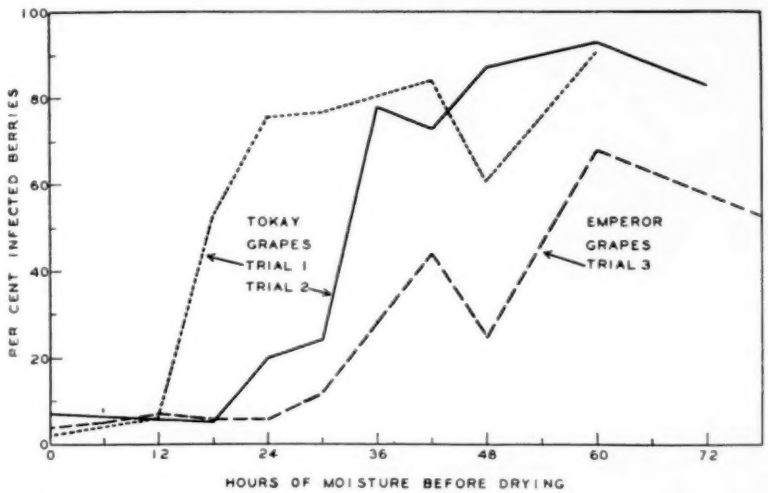

FIG. 1. Influence of temperature on length of wet period necessary to produce botrytis infection of Emperor and Tokay table grapes. Diurnal temperature ranges: Trial 1, 12° to 21° C.; trial 2, 2° to 19° C.; trial 3, 2° to 21° C.

The curve of each trial (Fig. 1) shows the percentage of infected berries 6 days after inoculation in each lot of 10 clusters kept wet for the indicated period of time. The 0 reading for hours of moisture shows the percentage of infected berries in lots dusted with conidia and kept dry through each test.

In trial 1, there was a rapid increase in the percentage of infected Tokay grapes between the 12th and 24th hr. of moisture on the berries when the minimum tmperature was 12° C. (Fig. 1). Approximately the same in-

crease occurred between the 18th and 36th hr. in trial 2, when the minimum was 2° C. Judging from these results, a minimum temperature difference of 10° C. caused a difference of 6 to 12 hr. in the length of the infection period.

The temperature range of trial 3 with Emperor grapes was the same as that of trial 2 with Tokays, yet the infection period was prolonged to 60 hr. Even at 60 hr., the percentage of infected Emperor berries was not so high as in the Tokays at 36 hr. This indicates a varietal difference in the degree of susceptibility to botrytis infection.

The temperature effect on infection could be determined only in a general way with this technique because of the diurnal fluctuation. Such effects were determined more accurately by incubating inoculated grapes at different constant temperatures.

Clusters of mature Tokay grapes were dipped in tap water, then sprayed with a spore suspension. They were immediately placed in individual waterproof bags, fastened securely to prevent evaporation. Dry clusters, used as controls, were dusted with dry spores and placed in individual manila bags. Each lot consisted of 7 clusters. One control lot and several sprayed lots were incubated at each of the four temperatures used: 12°, 16°, 20°, and 24° C. The lots were removed at intervals from the incubators and dried according to the schedule in table 1. After drying at

TABLE 1.—*Relation of hours of moisture to percentage of infection of Tokay grapes by Botrytis cinerea at four constant temperatures*

Hours of moisture	Temperature (° C.)[a]			
	12°	16°	20°	24°
	Percentage infected berries			
0	13	5	4	7
18	.....	.....	95	41
24	100	92	100	60
36	100	100	100	25
48	100	100	100	66
72	100	100	.....	84

[a] Temperature at 12° was constant to 2° C.; at the other temperatures, 1° C.

18° to 23° C. in an air stream from a fan for 3 to 6 hr., each lot was placed in a manila bag and returned to the incubator from which it had been withdrawn.

The percentage of infected berries for each lot was determined on the fourth day after inoculation (Table 1). In this experiment, less than 24 hr. of moisture were required to produce infection at each temperature. The low amount of infection at 24° indicates that this temperature may be above the optimum for infection of Tokay grapes, whereas the high amount at 12° C. indicates this lowest temperature is within the optimum range.

This experiment was repeated under slightly modified conditions. The control clusters were dipped in water and sprayed with a spore suspension

as were the others, then were immediately dried before being sacked. Clusters so treated were dry ½ hr. after dipping. The moist lots were removed from the different temperature chambers at 3-hr. intervals and dried for 3 hr.; they were re-sacked dry and returned to the incubators at the four different temperatures. The percentage of infected berries for each lot was determined on the fourth day after inoculation (Fig. 2). Infection was most rapid at 16° C., occurring in 9 to 18 hours. The infection periods (considered here the length of wet period necessary to produce appreciable infection) at 12° and 24° were about 3 hr. longer than at 16° and 20° C. The amount of infection produced after 21 hr. of incubation with moisture at 12° and 24° was greater than at 16° and 20° C. However, the data in-

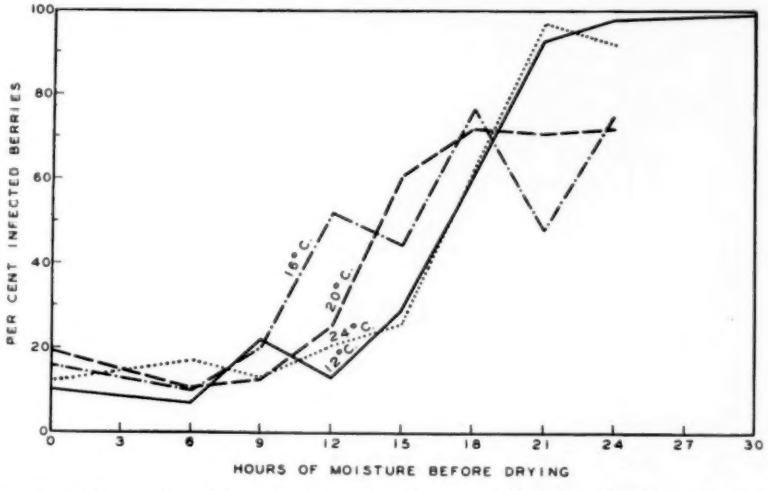

FIG. 2. Effect of moisture and temperature on infection of Tokay grapes with *Botrytis cinerea*.

dicate that the infection rate of botrytis is not materially different within the range of 12° to 24° C. In the vineyard it might be expected that infection would take place within 24 hr. after a rain, if spores are present on suitably mature grapes and the temperature is between 12° and 24° C.

These tests were repeated with Emperor grapes which had been in cold storage for 12 to 14 weeks. Some of the berries had slight sulphur dioxide gas injury at the base of the pedicels as a result of periodic fumigation to suppress the growth of storage-rot organisms. The same technique was used as in the previous experiment, except that before inoculation the lots of grapes were held in the different temperature chambers for 24 hr. to come to equilibrium with the respective temperatures. Also, the temperature of the dipping solutions was adjusted to that of the different temperature chambers. Each lot of seven clusters was placed in a waterproof bag after inoculation and immediately returned to the respective temperature chamber. At intervals, the lots were removed and dried at room temperature for 6 hr., and then were returned dry to the different temperatures.

The lots held at 20°, 25°, 30°, and 35° C. were removed 4 days after inoculation and the percentage of infected berries determined for each lot. Those held at 9°, 12°, and 16° were removed 6 days, and those at 3° and 6°, 10 days after inoculation (Fig. 3).

At 3° C., the infection period was 72 to 84 hr., indicating that botrytis spores were able to infect the grapes even at this low temperature. At progressively higher temperatures, the length of the infection period was reduced until at 16° only 12 to 18 hr. of moisture were required. At temperatures higher than 16°, the percentage of infected berries was less, and the infection period was slightly longer. The action of other microorganisms in the lots at 35° C. so obscured the symptoms that it was impossible to determine whether the rot was caused by *Botrytis*.

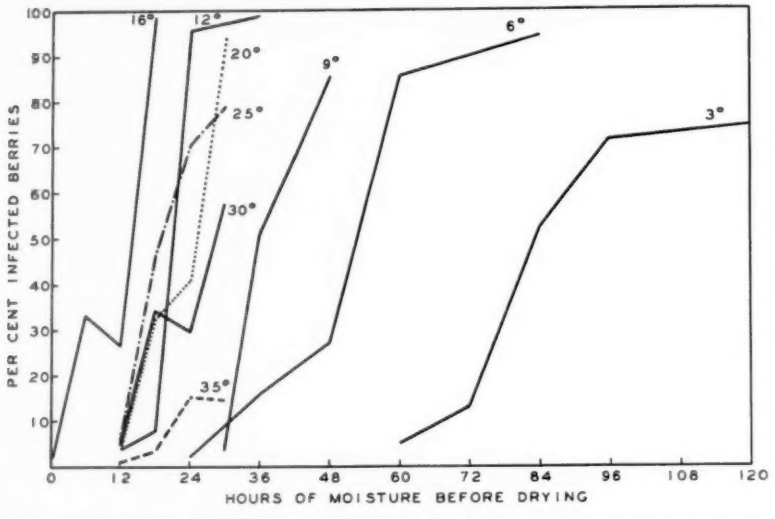

FIG. 3. The effect of different periods of moisture on the percentage infection of Emperor grapes by *Botrytis cinerea* at different constant temperatures. The only control lot shown for 0 hours of moisture is for that lot held at 16° C. The controls at the other temperatures showed less than 3 per cent infected berries, except the one held at 30° which showed 6.6 per cent.

The infection period was much shorter for Emperor grapes held at a constant temperature of 12° C. than for those in trial 3 of figure 1, held at 2° to 21° C. (average 12° C.). The lower temperatures of trial 3 probably account for the longer moisture period necessary to produce a high percentage of infection. However, it is possible that the storage of the grapes for 12 to 14 weeks may have rendered them more susceptible to infection than those in figure 1 which were used directly from the vineyard.

Maturity of fruit. Du Plessis (3) demonstrated that grapes harvested early in the season were less susceptible to botrytis rot than those harvested at later stages of maturity. During the course of the present investigation, it was noted that there appeared to be a consistent difference in the percentage of sugar in noninfected and infected berries from the same cluster

of grapes. The standard index of maturity of grapes for marketing purposes in California is the percentage of soluble solids of the berries. The refractive index of the total soluble solids in the extracted juice of the grape berries was measured by a Zeiss refractometer equipped to show percentage of sugar directly. Clusters of Tokay grapes, directly from the vineyard with naturally infected berries, were used. The average percentage of sugar of six noninfected berries of each cluster was compared with the average of six infected berries with very small lesions. The juice from the latter was extracted from noninfected tissue at some distance from any lesion. For the noninfected berries the range was 14.1 to 21.2 per cent, mean 18.2; for the infected berries, 16.2 to 23.3, mean 19.3 per cent. To test the difference, a paired experiment analysis was used, as described by Hayes and Immer (6, pp. 285–287). With 63 degrees of freedom a "t" value of 9.957 was obtained, whereas a "t" value of only 2.576 was necessary at the 1 per cent level of significance. Judging from the highly significant difference obtained, berries with a higher sugar content are more susceptible to botrytis infection than those lower in sugar.

DISCUSSION

In order to infect grapes, the conidia of *Botrytis cinerea* required a period of moisture, especially when the grapes were subsequently incubated in relatively dry air. The length of the moisture period necessary to bring about infection was demonstrated, though it was not shown whether this period included the stage when the germ tube penetrated the epidermis of the grape.

Vanderwalle (10) and du Plessis (4) found that wounded grapes were more susceptible to botrytis infection than unwounded grapes. The results of the present investigation, however, agree with the observations of Müller-Thurgau (8) that infection usually takes place through the uninjured skin. Microscopic injuries, where no evidence of any escaping juice could be detected, were not found by the writer to be essential courts of infection. On mature grapes, it may be that wounds are not so important as avenues of entrance for the fungus, as they are as sources of water and nutrients for the germinating spores.

The temperature range of infection is wide, including most temperatures encountered in storage and in the vineyard. The infection period for stored Emperor grapes incubated at a constant temperature of 12° C. was much shorter than that for fresh Emperors incubated at 2° to 21° C., averaging 12° C. The lower temperatures of the range probably account for the longer infection period. However, it is possible that the storage of the grapes for 12 to 14 weeks prior to the constant temperature experiment may have rendered them more susceptible to infection than those used fresh in the latter experiment.

The Tokay variety was more susceptible than the Emperor, as shown in figure 1, by a shorter infection period and higher incidence of infection.

This difference suggests one factor contributing to the better keeping quality of the latter variety.

SUMMARY

It was found that Tokay grapes were infected by *Botrytis cinerea* through the uninjured skin. Lenticels, insect punctures, and microscopic injuries were not essential courts of infection.

Grapes became infected from conidia only when the berries were kept wet after inoculation. The length of the moisture period necessary to produce infection varied with the temperature. Under conditions of diurnal fluctuations in temperature, infection of mature Tokay grapes required a moisture period of 12 to 24 hr. when the minimum temperature was 12° C.; with a minimum of 2° C., 18 to 36 hr. were required. Mature Emperor grapes required 30 to 60 hr. when the minimum was 2° C. This indicates a varietal difference in susceptibility to botrytis infection.

In the laboratory, the moisture period necessary for infection of stored Emperor grapes was 72 to 84 hr. at 3° C., and 12 to 24 hr. at 16° C. At temperatures progressively higher than 16° C., the amount of infection was less and the length of the infection period was slightly longer. At 35° C., little or no infection occurred.

The sugar content of noninfected berries was significantly lower than that of the healthy tissue of infected berries in the same cluster. Berries high in sugar were apparently more susceptible to botrytis infection.

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FACTORS AFFECTING SPORULATION OF DIAPORTHE PHASEOLORUM VAR. BATATATIS FROM SOYBEAN[1]

MARGARET BARTON TIMNICK,[2] VIRGIL GREENE LILLY, AND H. L. BARNETT

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Among the general conditions known to influence sporulation of fungi, the more important are: the complex and indefinite factors of natural media, composition and concentration of the nutrients of synthetic or semisynthetic media, temperature, and light. Duggar (4, chapter 27) reviewed the literature up to 1936 on the effect of light upon reproduction of the fungi. Klebs (8) noted that some fungi reproduce equally well in either light or darkness, whereas the reproduction of others may be either hindered or stimulated by light. Some fungi require alternate light and darkness for sporulation. It has been reported by Stevens (15), Smith (14), and Bailey (1) that irradiation with ultraviolet light stimulated the sporulation of *Glomerella cingulata* and species of *Fusarium*. Harter (5) and Lehman (9) found that total darkness inhibited formation of pycnidia by *Diaporthe sojae*. Few studies have been made regarding the interrelated effects of a number of factors, such as light, temperature, and composition of the medium. It is known, however, that such interrelated effects do exist with certain fungi and that these effects have frequently been ignored in experimental studies.

This paper reports the results of experiments designed to determine 1) the effects of nutritional and environmental factors on sporulation, and 2) the combined optimum laboratory conditions for maximum sporulation of *Diaporthe phaseolorum* var. *batatatis*. It represents a part of a more extensive study of conditions which favor or inhibit the production of spores by fungi.

MATERIALS AND METHODS

An isolate of *D. phaseolorum* var. *batatatis*, obtained from Dr. W. B. Allington, was used. It agreed in all major aspects with Wehmeyer's (16) description of this fungus.

Since the composition of the medium is one of the most important conditions influencing growth and reproduction of fungi, a number of natural and semisynthetic media were used, particularly in preliminary experiments:

1. Malt extract agar: 20 gm. Difco malt extract per liter.
2. Potato-glucose agar: 200 gm. sliced unpeeled potatoes were auto-

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claved with 500 ml. of distilled water for 10 min. at 15 lb. steam pressure. The autoclaved potatoes were filtered through cheesecloth, sufficient pressure being used to force some of the pulp into the filtrate. Twenty gm. each of glucose and agar were added and the volume made up to 1 liter.

3. Whole-wheat agar: 100 gm. Ralston whole-wheat cereal were added to 700 ml. of hot (about 90° C.) distilled water; the mixture was cooled 2 hr. and filtered through cheesecloth, enough pressure being used to force some starch into the filtrate. Distilled water was added to make 1 liter.

4. Soybean stems, pods, and seeds in 2 per cent water agar.

5. V-8-juice agar: Prepared according to Wickerham et al. (18).

6. Glucose-casein hydrolysate medium prepared according to Leonian and Lilly (11).

The basal synthetic medium used in the greater part of this work had the following composition:

KH_2PO_4, 1.0 gm., $MgSO_4 \cdot 7H_2O$, 0.5 gm.; nitrogen source to furnish 0.425 gm. N; carbon source, 10.0 gm.; distilled water to make 1000 ml. The pH before autoclaving was 5.0.

Twenty gm. of agar per liter were used with all of the above media except that specified as liquid. Media to which agar has been added are no longer strictly synthetic, for their composition is uncertain. However, in this work we obtained no evidence that agar contained metabolites which stimulated sporulation of this fungus. For most culture work 40 ml. per Petri dish were used. The vitamins, when added, were in the following amounts per liter: Thiamine, 100 μg.; pyridoxine, 100 μg.; biotin, 5 μg.; inositol, 5 mg.

The cultures were incubated at 25° C. in a constant-temperature room, where they received light from daylight fluorescent lamps for approximately 5 hr. each day. These conditions are hereafter referred to as the control conditions. They were chosen as a matter of convenience and were not based on any foreknowledge of their effects on this fungus.

Inoculum was either bits of mycelium from sporulating agar cultures, or minced mycelium cut in a Waring Blendor. Other details will be presented in connection with the individual experiments.

Replications of six to ten cultures were used, and all experiments were repeated at least twice. The data herein represent only a small portion of those obtained throughout the investigation.

REPRODUCTION ON NATURAL HOST MATERIAL

The fungus was grown on portions of the soybean plant since it is known that sporulation is often more abundant on natural host material than on synthetic media. Mature soybean seeds, pods, and pieces of mature stems were placed in Petri dishes with 20 ml. water agar, autoclaved, inoculated, and incubated at 25° C. After 20 days mature perithecia were present in great numbers on the portions of stems and pods above the surface of the agar. This proved to be the most favorable of all media for the formation

of perithecia. They were formed in greater abundance and more rapidly than on any of the synthetic media tried. No perithecia formed on seed even after 60 days. Perithecia also formed abundantly on sterilized stems and pods moistened with water, without the addition of agar.

Alpha spores (conidia) were also produced on the same stems and pods prior to the formation of perithecia. They oozed out of ruptured stromata in milky droplets, much like those illustrated for *D. phaseolorum* var. *sojae* by Welch and Gilman (17), who did not observe the production of asexual spores by *D. phaseolorum* var. *batatatis* under any condition. In our studies, these were the only conditions under which alpha spores were produced in abundance. Separate small pycnidia containing nearly all beta spores (stylospores) were present in small numbers on soybean stems, pods, and seeds as well as on a number of other media described later.

EFFECT OF CERTAIN NUTRITIONAL FACTORS

Any nutritional investigation of fungi must involve the study of vitamin requirements, and available sources of carbon and nitrogen. These factors often affect both growth and reproduction. On the other hand, some factors are known to affect sporulation without influencing the rate of growth. It was, therefore, necessary to determine whether this fungus was deficient for vitamins before sporulation studies could be undertaken on synthetic media.

Vitamins and growth. The fungus was grown in liquid glucose-casein hydrolysate medium (25 ml. per 250-ml. Erlenmeyer flask) to which thiamine, biotin, inositol, and pyridoxine were added separately and in certain combinations. After various periods of growth, two or three cultures from each condition were harvested and the dry weights obtained. Figure 1 plots the results from four conditions of one typical experiment. Initial growth was more rapid in the presence of inositol than in its absence, showing that the fungus is partially deficient for this vitamin. Although this deficiency is not great, it is distinct. Thiamine alone depressed the rate of growth.

Interrelated effects of vitamins and carbon source. Since the production of perithecia was affected by a number of interacting factors, the influence of each was tested under a number of conditions. It is more convenient to present the results of the vitamin and carbon experiments together, for the actions of these two factors in particular were found to be closely interrelated.

Various investigators have shown that reproduction, as well as growth, of some vitamin-deficient fungi is influenced by the supply of vitamins for which they are deficient. Experiments were set up to determine whether vitamins affect reproduction of this fungus, which is only slightly deficient. Other experiments were made at the same time to determine which carbon source was most favorable for the production of perithecia. A number of carbon sources were used, with asparagine as the nitrogen source (known

from preliminary experiments to be favorable), with and without the four common vitamins mentioned above. It was customary to begin each experiment with replicates of ten Petri-dish cultures. All contaminated cultures were discarded.

After 40 days the perithecial beaks (Fig. 2, A) were counted by means of a wide field stereoscopic microscope. The averages of the totals of five or six replicate cultures are in table 1.

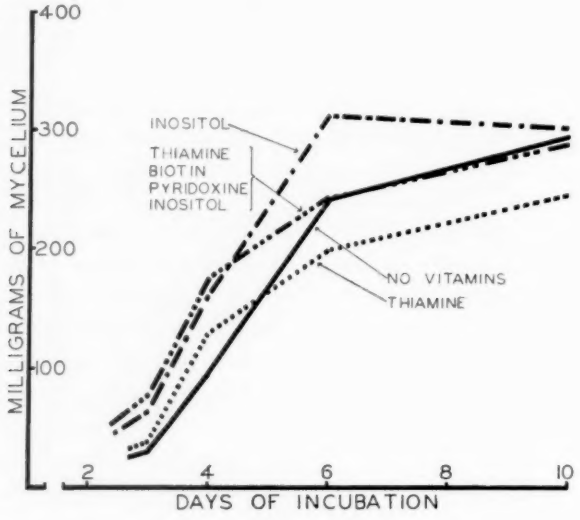

FIG. 1. Mycelial growth of *Diaporthe phaseolorum* var. *batatatis* in the presence of certain vitamins. Cultures were grown in 25 ml. liquid medium at 25° C. The more rapid rate of growth during the first 6 days indicates a slight deficiency for inositol.

The data in table 1 are from one experiment only, but repetitions gave comparable results. The addition of vitamins caused appreciable increase in sporulation only when lactose was the carbon source. On the other hand, these four vitamins had little effect on the numbers of perithecia formed when maltose and fructose were the carbon sources. When these four vitamins were added to media containing glucose and potato starch, perithecium formation was significantly decreased. Starch, lactose (Fig. 2, B), fructose, and mannitol were the most favorable sources of carbon for the formation of perithecia. Sorbitol, glucose, sucrose, and maltose were relatively poor for this purpose.

Nitrogen source. Similar experiments were made using different nitrogen compounds and lactose in the basal synthetic medium without vitamins. The following compounds were used: Ammonium sulfate, sodium nitrate, aspartic acid, glycine, urea, and asparagine. The greatest number of perithecia was formed on asparagine. Glycine and urea were also good sources of nitrogen. Few perithecia formed on sodium nitrate and aspartic acid, and none formed on ammonium sulfate.

Other nutritional factors. The amount of medium per Petri dish influenced reproduction. Trials with 10, 20, and 40 ml. of medium per culture were made. Perithecia were formed in greatest numbers on the cultures with 40 ml. Since 40 days were required for maximum sporulation, it is probable that the greater amount of medium kept the cultures from drying out and allowed more sporulation to take place. For this reason, a standard of 40 ml. per plate was adopted.

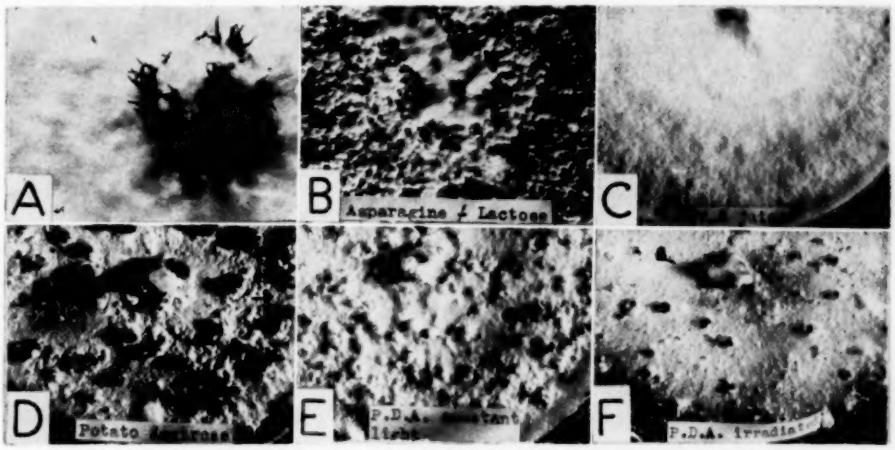

FIG. 2. Portions of cultures incubated under various conditions of nutrition and light. A. Typical normal stroma with protruding perithecial beaks, highly magnified. B. Stromata and perithecia on lactose-asparagine medium under control conditions. Note that the stromata are small and numerous. C. Culture on V-8-juice agar medium. No stromata are formed. D. Stromata and perithecia on potato-dextrose medium under control conditions. E. Stromata and perithecia on potato-dextrose agar when exposed to continuous intense light. Note decreased size of stromata and perithecial beaks. Compare with D and F. F. Stromata and perithecia on potato-dextrose medium when irradiated for five 1-min. periods. Both size and number of stromata are decreased.

The initial pH of the medium was not a limiting factor within the usual growth range. However, no perithecia formed when the initial pH of lactose-asparagine medium was above 8 or below 3. The greatest numbers were formed within the range of 4 to 6.

Certain natural media were better for sexual reproduction than lactose-

TABLE 1.—*Perithecia produced on media containing different sources of carbon, with and without vitamins. Cultures grown under control conditions for 40 days*

Carbon source	Average number of perithecia per plate	
	No vitamins added	Four vitamins added
D-Glucose	665	160
Sucrose	few	40
Maltose	525	665
Lactose	1615	3500
Sorbitol	none	none
Mannitol	1265	880
D-Fructose	1020	1210
Potato starch	4750	1140

TABLE 2.—*Perithecia produced after 40 days on some media of natural origin under different conditions of light and ultraviolet radiation*

Light treatment	Average number of perithecia per plate			
	Whole wheat	Malt extract	Malt extract + 2 per cent yeast extract	Potato-glucose
Control conditions	1575	1615	405	4140
Irradiated 1 min., 5 times	186[a]	990	1125	860
Continuous light	900[b]	435	none	420[b]

[a] From 70 to 80 stromata with no beaks, but with abundant asci and ascospores.
[b] Few mature asci and ascospores.

asparagine, while others were poorer. Of these, potato-glucose yielded the most perithecia under control conditions (Table 2). Whole-wheat extract and malt extract were equal to lactose-asparagine. The addition of 2 gm. yeast extract per liter of malt-extract medium caused a sharp decrease in number of perithecia. This may be due to an upset in the balance of nutrients or to an inhibitory substance in the yeast extract. No perithecia were formed on V-8-juice agar under any condition tested (Fig. 2, C).

EFFECT OF ULTRAVIOLET RADIATION

Preliminary experiments showed that ultraviolet irradiation of cultures favored formation of perithecia on glucose-casein hydrolysate medium; non-irradiated cultures on the same medium produced no perithecia. In other experiments, to determine the most effective dosages and number of exposures on cultures of different ages, cultures in Petri dishes with lids removed were placed 10 in. below a 15-watt G. E. germicidal lamp (2537 Å). Five 1-min. exposures (one every other day) were more effective than longer or more frequent exposures. On glucose-casein hydrolysate medium, cultures 2, 4, and 9 days old when first irradiated produced an average of 450, 390, and 16 perithecia, respectively. Repetition of this experiment gave the same general results, which agree with those of Stevens (15) on the effects of irradiation on *Glomerella cingulata*. Other experiments, not reported in detail, showed that these exposures were near optimum for the production of perithecia on this medium.

When the fungus was grown on whole-wheat, malt-extract, and potato-glucose media (Fig. 2, D, E, F), ultraviolet radiation decreased the number of perithecia (Table 2) and suppressed the development of perithecial beaks. Longer exposures prevented the development of normal perithecial beaks but did not inhibit the formation of mature ascospores. These results indicate that the same dosage of ultraviolet radiation may favor or inhibit the formation of perithecia depending upon the medium.

EFFECT OF LIGHT AND DARKNESS

Under the control conditions selected for these investigations the cultures received alternating light and darkness. Facilities did not permit a more rigid control of light conditions at this time.

To determine whether increased light intensity and duration would affect perithecium production, cultures on certain media were exposed to continuous light (75 foot-candles) from a daylight fluorescent lamp. A portion of the results is in table 2, in order to compare the effects with those of ultraviolet radiation. Both treatments resulted in fewer perithecia (on most media) than were produced under control conditions (Fig. 2, D, E, F).

Our best synthetic medium, lactose-asparagine, was used in a further study of perithecium formation (Table 3).

TABLE 3.—*Perithecia and relative abundance of ascospores produced by cultures on lactose-asparagine medium under different light conditions*

Light conditions during incubation	Average number of perithecia per plate	Ascospores
Control (alternate light and darkness)	1500	Abundant
Continuous light	400	Few
Continuous light, 9 days; darkness, 31 days	250	Abundant
Continuous darkness	3	Abundant
Darkness, 9 days; continuous light, 27 days	600	Many

Because of the morphology of the fungus, it was impossible to separate all of the ascospores from the perithecia so that the spores could be counted with any degree of accuracy. For this reason only estimates of the relative abundance of ascospores are reported. This was determined by microscopic observations of crushed perithecia.

Continuous light permitted the development of stromata and perithecia, but delayed the development of ascospores. Continuous darkness allowed the formation of only a few perithecia, but these produced abundant mature ascospores. Alternate light and darkness, to which the control cultures were exposed, was much more favorable for the production of perithecia, asci, and ascospores than was either continuous light or darkness. An intermediate number of perithecia was produced after an extended period of light followed by darkness, or darkness followed by light. Thus, exposure to alternating short periods of light and darkness, such as those occurring in nature, is probably near optimum for the production of ascospores.

PRODUCTION OF PYCNIDIA

The observations reported here were made on the same cultures used for the study of the production of perithecia and ascospores. Since the formation of pycnidia containing nearly all beta spores (with only an occasional alpha spore) is not believed to be of great significance in the reproduction of the fungus, only a brief report on this phase will be given. The pycnidia were small, separate, and partially imbedded in the medium in contrast to the indefinite stromatic structures on soybean stems and pods from which the alpha spores oozed in great numbers. The beta spores were 8 to 16 μ long and somewhat smaller than those described by Wehmeyer (16) for this fungus. Many of the smaller spores are believed to be intermediate between the two spore forms.

Pycnidia were produced on a number of media, including: Lactose-asparagine, lactose-urea, potato-glucose, malt extract, soybean stems, pods, and seeds. Both continuous light and ultraviolet radiation greatly increased the number of pycnidia on potato-glucose medium. Pycnidia formed on V-8-juice agar irradiated with ultraviolet light, whereas none formed on the control. Continuous darkness inhibited the formation of pycnidia.

DISCUSSION

Kauffman (7) stressed the need of a favorable internal environment before reproduction can take place. He held that the internal environment of the cells of a fungus is largely controlled by external conditions such as light, temperature, and nutritional factors, and that by properly manipulating the external environment it is possible to bring the internal environment of a fungus into the condition which allows reproduction. While these broad views admit a general application to all fungi, the specific application rests upon the detailed study of each fungus investigated. The present study emphasizes the complexity of the interactions between the various factors. It clearly shows that sporulation is affected by a number of conditions and that one condition may modify the influence of another.

The experiments showed that, while *D. phaseolorum* var. *batatatis* is partially deficient for inositol, the influence of this and other vitamins on the production of perithecia was variable. Sporulation was increased on certain media and decreased on others. Synthetic media solidified with agar (semisynthetic media) were, in general, poorer for the production of perithecia than were natural media. Pods and stems of the host plant were the best medium for sporulation.

Ultraviolet irradiation of the mycelium enabled the fungus to form perithecia and ascospores on certain media, such as glucose-casein hydrolysate, which were otherwise unfavorable. The internal conditions necessary for reproduction were attained on this medium only after the mycelium had been irradiated. It is known that this treatment kills a portion of the mycelium. It is believed that some of the products of the dead autolyzing cells may furnish the necessary nutrients or stimulants for reproduction. This is also in line with the common observation that some fungi reproduce only after maximum growth has been attained and autolysis has begun. There is some evidence that autolytic products favor reproduction of certain other fungi (12).

It may be assumed that this fungus, when grown on lactose-asparagine or potato-glucose medium, has available or is able to synthesize all of the compounds necessary for abundant sporulation. On these media irradiation caused a decrease in numbers of perithecia, in contrast to the stimulatory effect described above. It should be emphasized that the primary effect of ultraviolet irradiation is the killing of the exposed cells. Its action may be

harmful rather than beneficial, if the fungus is grown on a medium containing all of the nutrients necessary for abundant sporulation.

Light is required for the reproduction of numerous fungi and is beneficial to others, but little or nothing is known about the specific metabolic processes modified by light. Coons (2) attributed the favorable effect of light on pycnidial formation by *Plenodomus fuscomaculans* in part to oxidation, for he found that the effect of light was replaced to some extent by hydrogen peroxide and other oxidizing agents. Leonian (10) found that increased temperature of incubation partially replaced light, as a stimulus for the production of pycnidia by some Sphaeropsidales.

Houston and Oswald (6) found that light, preferably daylight, was necessary for *Helminthosporium gramineum* to produce conidiophores and conidia on agar. When diseased leaves were brought into the laboratory and cultured, the mycelium was capable of producing conidiophores and conidia in the dark. This suggests that the composition of the medium is a factor in determining the need for light.

Fewer perithecia are produced by *Diaporthe phaseolorum* var. *batatatis* when the cultures are exposed to continuous light than when they are under alternating light and darkness. Cultures kept in darkness formed only a few perithecia, but these contained many mature asci and ascospores. There is some evidence that the effects of light vary with the intensity of the light. Light apparently is essential for the maximum formation of perithecia, but is not needed while ascospores are forming; intense light is somewhat inhibitory at this time.

External factors also affect the type and amount of asexual spores formed. Pycnidia containing mostly beta spores were produced in variable numbers on all media used, and under different conditions of light and ultraviolet radiation. The finding that total darkness was unfavorable for pycnidial formation confirms the results of Harter (5) and Lehman (9) with *D. sojae*. The fact that the beta spores varied in size, depending upon the medium on which they were produced, is not surprising in the light of the work of Das Gupta (3) on *Cytosporium ludibunda* and that of Nitimargi (13) on *Phomopsis, Cytosporina,* and *Diaporthe*. Alpha spores were produced in abundance only on soybean stems and pods. It is worthy of note that Welch and Gilman (17), who cultured an isolate of the same fungus, failed to observe either type of asexual spore. It seems that this can best be explained on the assumption that our isolate differs from theirs in its requirements for asexual sporulation.

SUMMARY

The present study has shown that *D. phaseolorum* var. *batatatis* is partially deficient for inositol and that the effect of inositol and other vitamins on sporulation varied with the source of carbon in the medium. Production of stromata and perithecia occurred on synthetic media solidified with agar as well as on soybean stems and pods, although the natural-host media

permitted greater numbers of perithecia to form, in a shorter period of time. Ultraviolet irradiation either stimulated or inhibited the formation of perithecia, depending on the medium used. Maximum production of ascospores occurred when cultures received periods of both light and darkness. Continuous total darkness was unfavorable for the formation of perithecia. Small separate pycnidia containing almost entirely beta spores were formed on most media, and their production on certain media was increased by exposure to ultraviolet irradiation. They failed to form in continuous darkness. Alpha spores were produced in abundance only on soybean stems and pods.

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THE CHEMOTHERAPEUTIC CONTROL OF FUSARIUM WILT OF CARNATIONS

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The chemotherapeutic approach to the control of plant diseases is a rapidly advancing realm of plant pathology. Through highly controlled assays for therapeutic activity[1] compounds may be selected which, when absorbed by the plant from the soil, will stamp out incipient infections.[2] In such experiments plants are grown in sand on Hoagland's solution and in a controlled environment, and the value of these chemotherapeutants in commercial practice has been uncertain. The purpose of this note is to indicate the value of such treatments under commercial greenhouse conditions,[3] to point out that the losses prevented have not previously been controllable, and particularly to describe two compounds with very high chemotherapeutic efficiency.

Traditionally, the control measures against the soil-borne wilts and root rots of carnations have been entirely preventive. Recommended control measures often are inadequate and losses from these diseases may be high even when the grower has taken all possible precautions. Chemotherapy appears to be a useful combative procedure at such a time. When the first sign of disease appears in a greenhouse, treatment with effective compounds may reduce losses.

2-Norcamphane methanol,[4] 4-chloro-3,5-dimethyl phenoxy ethanol,[4] and 8-quinolinol sulfate[5] are all three useful for this purpose. Under the code numbers 1207 and 1182 the first two have been shown to have chemotherapeutic efficiency against Fusarium wilt of tomato,[2] and the methods by which these experiments are carried out have been briefly described.[1] Suffice it to say here that these compounds, when applied in water solution to sand in which plants are growing, enter the roots and become distributed in the plant. Under proper circumstances the first two can protect the tomato plant completely against a most severe inoculation with *Fusarium*

[1] Chapman, R. A., A. E. Dimond, and E. M. Stoddard. Assaying chemotherapeutants in the greenhouse. (Abstr.) Phytopath. 40: 4. 1950.

[2] Horsfall, J. G., A. E. Dimond, E. M. Stoddard, and R. A. Chapman. Chemotherapy for controlling plant diseases. Proc. 2d Internat. Congr. Pl. Prot., London. In press. 1949.

[3] We are indebted to S. F. Carabetta and Sons of Meriden, Connecticut, carnation growers, in whose greenhouse these studies were made.

[4] These compounds were generously provided by Carbide and Carbon Chemicals Division, Union Carbide and Carbon Corporation, 30 East Forty-second Street, New York 17, N. Y.

[5] Fron (Fron, G. La maladie de la fusariose des Oeillets, Rev. Pat. Veg. 23: 131–144. 1936) reported similar use in 1932 of 8-quinolinol sulfate for combating *Fusarium dianthi* on carnations. He showed that soaking cuttings from healthy plants for 12 to 18 hr. in a 1: 20,000 solution before rooting, then spraying plants with 1: 20,000 to 1: 50,000 solutions, reduced losses from 80 per cent to 20 per cent in susceptible varieties and that the loss on most varieties was a mere 3 to 5 per cent.

lycopersici. 8-Quinolinol sulfate, applied in this way to tomato, is by no means so efficient as the other two.

In the present study these compounds were applied to the soil in greenhouse benches as water solutions at nonphytotoxic concentrations as follows: 8-quinolinol sulfate 1:4000, 2-norcamphane methanol 1:16,000, 4-chloro-3,5-dimethyl phenoxy ethanol 1:64,000, and copper-8-quinolinolate 1:4000. The experiments were conducted upon carnations of Miller's Yellow variety, which is highly susceptible to losses from *Fusarium.* Cuttings rooted from apparently clean stock were grown in sterilized soil and benched in the greenhouse during the first week of July, 1949.

Treatments were begun on July 19, 1949, and were made twice a week for a month, after which applications were made at weekly intervals. Commencing August 30, 1949, and for 2 weeks following, the concentration of chemotherapeutant was doubled. In all instances 50 ml. of solution were applied to the soil around each plant (approximately 12½ gal. solution per 100 sq. ft. of bench surface). Plots consisted of lots of approximately 100 plants. Treatments were discontinued on September 15.

During the course of the experiment, the number of plants which died of Fusarium was recorded from time to time. Such plants were cultured sufficiently frequently to determine that Fusarium was the cause of death in the vast majority of cases.[6]

Results of the experiment are summarized in figure 1, in which is plotted the percentage of plants lost by disease against the date of reading. It is interesting that copper-8-quinolinolate[7] and the check were identical in performance, whereas compound 1207 (2-norcamphane methanol) and 8-quinolinol sulfate showed almost identical protection. Compound 1182 (4-chloro-3,5-dimethyl phenoxy ethanol), however, gave slightly superior protection from disease during the period when it was being applied (shown by the dark horizontal bar in the figure), but this superiority disappeared later, probably indicating breakdown of the compound in the soil or in the plant.

There is ample evidence that both 2-norcamphane methanol and 4-chloro-3,5-dimethyl phenoxy ethanol are absorbed into the plant when the compound is applied to soil in which plants are growing. The former compound has a characteristic odor, and foliage of both tomatoes and carnations treated in the manner described takes on this odor shortly after applications are made. Flowers do not take on this odor, however. 4-Chloro-3,5-dimethyl phenoxy ethanol, when applied to tomatoes, produces a shoestring symptom on tomato foliage which is strongly reminiscent of 2,4-D injury, but this symptom is not produced upon carnations. In tomatoes, evidently the material is rapidly broken down in the plant, since tissues de-

[6] No extensive effort was made to distinguish by culturing whether the damage was caused by *Fusarium dianthi,* cause of wilt, or by *Fusarium culmorum,* cause of root rot. In diseased plants, vascular discoloration always occurred and maceration of the roots was infrequent; on this basis the principal pathogen involved was judged to be *Fusasarium dianthi.*

[7] Supplied for these studies by Monsanto Chemical Co., St. Louis, Missouri.

veloping a short while after applications are made have no such abnormality.

The formative action of 4-chloro-3,5-dimethyl phenoxy ethanol is of practical interest. While producing no effects upon leaves of carnations, treated plants in experimental plots made noticeably denser growth than plants in other plots. As a measure of this effect the number of "breaks" or terminal growing points per stem were counted. These were, for the several plots: check, 3.57 breaks per stem; copper-8-quinolinolate, 3.45; 8-quinolinol sulfate, 3.41; 2-norcamphane methanol, 3.34; and 4-chloro-3,5-

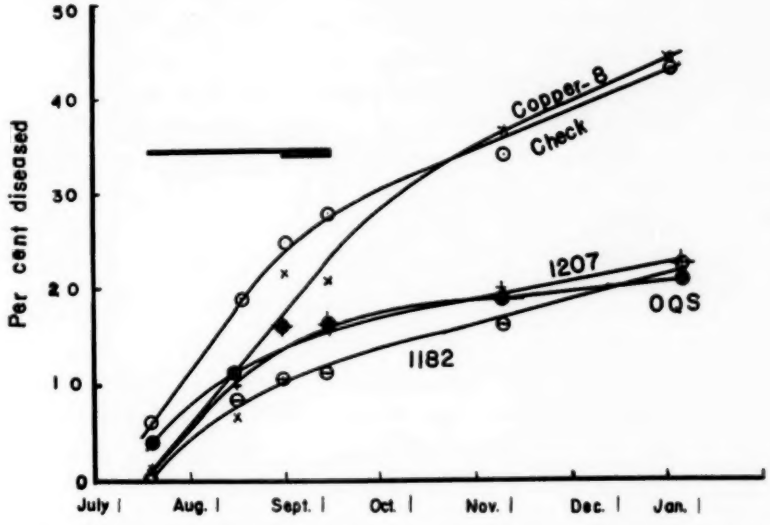

FIG. 1. Incidence of Fusarium wilt on carnations in plants treated chemotherapeutically. Horizontal bar shows period of application and concentration at which compounds were applied. All applications except the last two were made at relative concentrations stated in table 1 and the last two were at twice that stated. Treatments were as follows: Copper-8: copper-8-quinolinolate; 1207: 2-norcamphane methanol; 1182: 4-chloro-3,5-dimethyl phenoxy ethanol; and OQS: 8-quinolinol sulfate.

dimethyl phenoxy ethanol, 4.78. It will be noted that the last-named compound was the only one causing a material change in number of breaks per stem. Analysis by the Wilcoxon ranking method[8] indicates that among treatments, significant differences probably occur ($P = 0.04$).

4-Chloro-3,5-dimethyl phenoxy ethanol when applied to cuttings has likewise increased the rate of rooting and the number of roots formed. This effect is erratic, however, and the details of the procedure have not yet been satisfactorily worked out. Highly favorable effects of treatment were noted in certain experiments, whereas the same rate of application proved injurious in others.

During the growing season of 1950 the chemotherapeutic value of 8-quinolinol sulfate and of 4-chloro-3,5-dimethyl phenoxy ethanol and 2-nor-

[8] Wilcoxon, Frank. Some rapid approximate statistical procedures. Amer. Cyanamid Co., Stamford, Conn. 12 pp.

camphane methanol was again confirmed in a commercial greenhouse, this time on the variety Hercules. At the strength specified and in the manner already described, these materials were applied at weekly intervals for about 4 months to rooted cuttings in flats. At the end of this time they were benched, the diseased plants being discarded. Of 3026 cuttings treated with 2-norcamphane methanol, 2.9 per cent were diseased; of 1530 cuttings treated with 4-chloro-3,5-dimethyl phenoxy ethanol, 3.2 per cent were diseased. There were 1575 cuttings treated with 8-quinolinol sulfate, and of these 4.0 per cent were diseased. In contrast, 50.6 per cent of the 350 untreated control cuttings were diseased. Apparently, then, chemotherapeutic techniques are ready for commercial use in control of Fusarium wilt of carnations.

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INDICATIONS OF A NEW-WORLD ORIGIN OF TOBACCO-MOSAIC VIRUS

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World-wide distribution has been attained by some viral diseases of plants, especially by those of crop or ornamental plants that are propagated vegetatively and by those of which the etiological agents are transported in seeds or dried tissues. Relatively narrow geographic limits characterize many others, however, and it is probable that even those most widely distributed existed once in more restricted habitats than they have now.

It seems obvious that wherever a disease has existed for a long time, the most susceptible of its host plants will have been depressed in competitive vigor. Any less affected mutants from these host plants will have tended to supplant, or will have supplanted, the original representatives of the species. For continued existence of the causative agent, however, some plants must remain susceptible enough to maintain an adequate reservoir of the disease.

It is, of course, a serious question whether we can now determine the original endemic areas for viral diseases that have become widely distributed. Nevertheless, on the hypothesis that resistant species or varieties within generally susceptible groups of plants will be most in evidence where a disease has existed longest and thus has had most opportunity to discourage highly susceptible types, it has been evident for some years that rough guesses could be made of the geographical origins of several viral diseases. The substantial amount of information that has become available in recent years concerning tobacco mosaic has encouraged the present attempt to deal in some detail with the question of its area of original endemicity.

Tobacco-mosaic virus (*Marmor tabaci* H.) is found infecting plants today in every continent. This wide distribution is probably in large part a result of human activities that have extended its effective reservoir. For more than three centuries tobacco products, derived in part from infected plants, have been shipped from one country to another continually. Naturally, some of these products have reached farms where susceptible crops were cultivated and have contaminated the hands and tools of agricultural workmen. So stable is the virus of tobacco mosaic that products containing it ordinarily remain infective until they can reach and be utilized in their countries of destination, however distant. As a result of this, susceptible plants in widely separated parts of the world become diseased each year through slight abrasive contacts in the presence of viruliferous dusts or of liquid extracts derived from infective materials.

Originally, tobacco-mosaic virus probably was confined to a limited ter-

ritory for lack of the mobile reservoir of infective materials constituted today by tobacco products. No written history exists to indicate the boundaries of its incidence in ancient times, but nature has preserved records from which, in accordance with the hypothesis outlined above, the approximate extent of its prehistoric habitat may, perhaps, be deduced.

The solanaceous genus *Nicotiana* L. lends itself well to an investigation of the probable original habitat of tobacco-mosaic virus, because it furnishes especially good material for studying the geographic distribution of resistant and susceptible host types (8, table 1). Its constituent species, natives of the two American continents and of Australia, have all proved susceptible to experimental infection, insofar as they have been tested, but some possess an effective resistance of one sort or another in the field. This resistance may, in some cases, approach a field-immunity. In explanation of this term it may be pointed out that, whereas individual-immunity requires that each plant be insusceptible, a practical field-immunity does not require insusceptibility of individuals but only a response on infection inadequate to support spread of disease from plant to plant in the field. That is, a species may be so highly resistant as to be almost immune with respect to a particular disease, even though each of its plants is potentially susceptible to infection, provided that its type of response greatly hinders or entirely prevents spread of virus from plant to plant. The field rather than the individual is protected. Hypersensitive plants, for example, tend to be field-immune in the absence of frequent infection from outside sources, because little virus develops before death of infected tissues and the dead tissues are not readily broken to liberate this virus. Transmitting agencies ordinarily fail to operate effectively under these conditions. As a result, disease does not continue to spread, even if begun, though all individual plants may be highly susceptible to infection. Other characteristics tending to limit the amount of virus that is produced in infected plants and that is available for transfer to new hosts may play a somewhat similar rôle in limiting or preventing spread of virus from plant to plant.

The species of *Nicotiana* most resistant to tobacco-mosaic disease in the sense that they would constitute very poor reservoirs of virus in nature are those that respond to infection in a hypersensitive manner. These resistant species include *N. glutinosa* L., *N. repanda* Willd., *N. rustica* L., and *N. langsdorffii* Weinm. In addition, this type of resistance characterizes some lines of *N. undulata* Ruiz & Pavon and of *N. alata* Link & Otto, as will be explained presently. The status of *N. acuminata* (Grah.) Hook is in doubt, though one strain received under this name has proved hypersensitive (8).

The four definitely hypersensitive species may be assumed to possess field-immunity as defined above. Examination of specimens in several herbaria has made it evident that *N. glutinosa* is a native of Peru, *N. repanda* of Mexico, *N. rustica* of Ecuador and Peru, and *N. langsdorffii* of Brazil.

The partly field-immune species, that is, those that show hypersensitivity in some lines but not in all, are from countries bordering the general area characterized by this type of resistance. Thus a strain of *N. undulata* Ruiz & Pavon from Bolivia was hypersensitive but a strain from Peru was not. Some lines of *N. alata,* a native of Brazil and Uruguay, are hypersensitive; others merely mottle when infected. A line of unknown origin studied under the name of *N. acuminata* proved hypersensitive, whereas the doubtfully distinct species *N. caudigera* Phil. of Chile did not. Whether the plants earlier tested as *N. acuminata* really represented the species of that name usually regarded as Chilean is not clear, because some collections have been attributed to California and the State of Washington, far from the supposed Chilean home of the species. Because of the uncertain origin of the seed stocks regarded earlier as representative of *N. acuminata* (8), new lots of seed of known origin were obtained through the kindness of T. H. Goodspeed and R. E. Clausen of the University of California. One lot from California and one from the State of Washington represented North American sources; three viable lots from Chile represented South American material. None of these responded in the same way as the earlier studied line of unknown origin. All showed chlorosis and chlorotic mottling after experimental inoculation. Only the Chilean lots are represented on the map, figure 1, because it is not clear how long the species has occurred in North America.

The species of *Nicotiana* that are most susceptible to spread of infection in the field are those that tend to maintain a concentrated reservoir of tobacco-mosaic virus among themselves when infected. These are the species that respond by conspicuous chlorosis and chlorotic mottling. This group includes *N. nudicaulis* S. Wats. of Mexico and all tested species that are natives of the western United States, namely *N. palmeri* A. Gray, *N. bigelovii* S. Wats., *N. clevelandii* A. Gray, and *N. trigonophylla* Dun.; *N. rustica,* a hypersensitive species, has been reported from New Mexico but its occurrence there was regarded by Beinhart (2) as due to recent introduction. It includes also a number of species that are natives of Argentina, Uruguay, Paraguay, and Chile; these are *N. sylvestris* Spegaz. & Comes of Argentina, *N. otophora* Griseb. of Argentina, *N. bonariensis* Lehm. of Uruguay, *N. longiflora* Cav. of Argentina, Paraguay, and Uruguay, *N. solanifolia* Walp. of Chile, and *N. caudigera* of Chile. Finally it includes all tested Australian species. These Australian species were not studied in the preliminary investigation (8) but were tested to supplement this information for the present purpose; they are *N. benthamiana* Domin, *N. debneyi* Domin, *N. eastii* Kostoff, *N. exigua* Wheeler, *N. gossei* Domin, *N. maritima* Wheeler, *N. megalosiphon* Heurck & Muell., *N. occidentalis* Wheeler, *N. rotundifolia* Lindley, *N. suaveolens* Lehm., and *N. velutina* Wheeler.

Species of the most resistant (hypersensitive) group are, as has been mentioned, natives of Peru, Bolivia, Ecuador, Brazil, Uruguay, or Mexico. Species of the most susceptible (mottling-type) group are natives of the

western United States, Mexico, Argentina, Chile, Paraguay, and Uruguay in the Western Hemisphere, and of Australia in the Eastern Hemisphere. Thus the highly susceptible species are in general from areas north, west, and south of the central homelands of those that are highly resistant. This

FIG. 1. Map of the two American continents and Australia (inset), showing habitats of the 36 tested *Nicotiana* species and their types of response to infection by tobacco-mosaic virus. The approximate habitat of each species is indicated by a representative location at which it has been collected in nature. Fully susceptible mottling-type species are indicated on the map by circles; hypersensitive species, by squares; species of which some lines are hypersensitive but others of mottling type, by circles within squares; tolerant species, by triangles; and species that severely restrict growth of chlorotic areas in mottled leaves, by diamonds. Numbers indicate species: 1, *N. acuminata;* 2, *N. alata;* 3, *N. benthamiana;* 4, *N. bigelovii;* 5, *N. bonariensis;* 6, *N. caudigera;* 7, *N. clevelandii;* 8, *N. debneyi;* 9, *N. eastii;* 10, *N. exigua;* 11, *N. glauca;* 12, *N. glutinosa;* 13, *N. gossei;* 14, *N. langsdorffii;* 15, *N. longiflora;* 16, *N. maritima;* 17, *N. megalosiphon;* 18, *N. nudicaulis;* 19, *N. occidentalis;* 20, *N. otophora;* 21, *N. palmeri;* 22, *N. paniculata;* 23, *N. plumbaginifolia;* 24, *N. raimondii;* 25, *N. repanda;* 26, *N. rotundifolia;* 27, *N. rustica;* 28, *N. solanifolia;* 29, *N. suaveolens;* 30, *N. sylvestris;* 31, *N. tomentosa;* 32, *N. tomentosiformis;* 33, *N. trigonophylla;* 34, *N. undulata;* 35, *N. velutina;* and 36, *N. wigandioides.*

Records of collection were made available through the kindness of members of the staffs of the Gray Herbarium, United States National Herbarium, Brooklyn Botanic Garden, and the New York Botanical Garden.

evidence would seem to imply that the original habitat of tobacco-mosaic virus was within an area of the New World, centering about some part of Peru, Bolivia, or Brazil.

It may well be asked in what plant or plants effective reservoirs of the virus could have been maintained in the geographic area characterized by highly resistant species. A multiplicity of hosts of varying effectiveness as reservoirs probably existed. We need not, of course, assume that a reservoir host, or hosts, would fall taxonomically within the genus *Nicotiana* or even within the family Solanaceae; neither, however, is there any known reason for assuming the contrary. It is improbable that any plant of hypersensitive type would have functioned effectively as a reservoir, because death of infected tissues would severely limit availability of virus and greatly hamper transmission to new hosts. Nevertheless, it would seem that the habitat of an effective reservoir plant or plants originally must have matched or exceeded the range in which hypersensitivity developed, because such a plant, or plants, would be necessary to furnish repeated infections if natural selection for resistance were to be induced. It is, of course, improbable that any plants of typical mottling type would long have served as reservoirs without themselves drifting toward resistance by natural selection, in view of their probable poorer growth when infected and the competitive population pressure of other plants.

Three Nicotiana species, *N. glauca* Grah., *N. raimondii* Macbride, and *N. wigandioides* Koch & Fint, are neither hypersensitive nor much mottled when diseased and would share with the hypersensitive group an effective resistance to widespread damage. These three are, in fact, tolerant species and may act as symptomless carriers. *N. raimondii* may show chlorotic mottling or chlorotic spotting at times; at other times it may be symptomless and a carrier host. *N. wigandioides* tends to restrict virus to the inoculated leaf without obvious injury to infected tissues. The reactions of these two species have not been studied very fully, but *N. glauca* is better understood. It is extremely tolerant, varying from symptomless to faintly mottled when infected experimentally or in nature, and is very little reduced in rate of growth, or in ability to set seed, by tobacco-mosaic disease. It shares with typical mottling-type species the ability to maintain a substantial reservoir of virus, though a tendency to restrict virus to leaves other than the youngest seems to be essential to its tolerance and moderately reduces its effectiveness as a reservoir. It is now more widely distributed than any other wild species of *Nicotiana*, occurring for example in Argentina, Chile, Peru, Bolivia, Venezuela, Paraguay, Uruguay, Mexico, and California, but may have been native originally in a more limited area, perhaps in Argentina and Uruguay. Until another equally well-adapted reservoir host is recognized, it may be taken as an attractive hypothesis that *N. glauca* played an important rôle in maintaining tobacco-mosaic virus in its original habitat. *N. raimondii* and *N. wigandioides* may have played supplementary rôles locally, in Peru and Bolivia, respectively.

Three additional species of *Nicotiana, N. tomentosiformis* Goodspeed of Bolivia, *N. tomentosa* Ruiz & Pavon of Bolivia and Peru, and *N. paniculata* L. of Peru, show responses that might tend to limit spread from infected to healthy plants in the field, while permitting the species to act as somewhat effective reservoirs. These are the three wild species that are known to produce conspicuous enations when infected. The enations, or abnormal outgrowths of leaf tissue (9), appear to be formed in infected foliage as a result of nearly normal growth of green areas but sharp suppression of some chlorotic areas in mottled leaves. Since chlorotic areas of mottled plants are ordinarily the main sources of virus for spread to neighboring plants, a mechanism that leads to partial suppression of these may be comparable in its total effect to that leading to exclusion of virus from youngest tissues in plants that tend to be symptomless carriers.

Figure 1 is a map of the distribution of all naturally occurring *Nicotiana* species that have been tested by experimental inoculation with tobacco-mosaic virus. For each species, a representative site at which the plant has been found growing in nature is indicated. Only three species have such wide distributions as to make this selection of a representative location difficult. These are *N. glauca* and *N. acuminata,* as already described, and *N. plumbaginifolia,* which seems to have been collected most commonly from Bolivia, though also from Argentina and Mexico. *N. tabacum* L. has not been found occurring in nature except as an escape from cultivation and on this account is not included. It must be borne in mind that species found to have one type of response to infection might show other types of response also if other collections were tested. In the species of wide distribution this would affect the accuracy of the map whenever the tested lines came from locations other than that plotted as most representative for the species.

The map shows that resistant *Nicotiana* species of all types are located within a larger area occupied by the genus as a whole. The area characterized by more than one kind of resistance is even more restricted and is located centrally in the general area of resistance. This smaller area, which involves Peru and parts of the adjacent countries, seems to fulfill the earlier-mentioned expectation that somewhere in the world, namely, in the original habitat of tobacco-mosaic virus, intensive competition in the presence of the induced disease would have discouraged field-susceptible types and encouraged various resistant types within a generally susceptible group of plants. The principle that resistant species or varieties would be expected to be most in evidence where the disease has existed longest would suggest that the outline of the area displaying several resistances may be taken as indicating more accurately than any other now-recognized criterion the probable original habitat of tobacco-mosaic virus in prehistoric times.

It has been suggested that the center of diversity with respect to types of resistance in *Nicotiana* is approximately the center of diversity for the genus as a whole. This may be a coincidence, or may possibly point to a long-term dependence of the virus on this genus as including one or more impor-

tant reservoir species. If the genus had met the virus only in North America, or only in Australia, as a result of spread of the virus from plant-host reservoirs in other genera of plants, a very different picture would have been anticipated in the distribution of resistances in present-day species of *Nicotiana*. The evidence from this genus at present seems most consistent with a hypothesis of such a New-World origin of tobacco-mosaic virus as is suggested in the preceding paragraph.

It has been suggested also that rapidity of spread of disease in each region would have much the same effect on evolution of resistance in hosts as would length of time of association between virus and host plants. That is, amount of exposure, rather than duration or rate, would determine effectiveness. Certainly a distorting effect would be anticipated from differences in rapidity of spread and one might expect that if two centers of resistance existed they could have as explanation the two distinct causes. A single center of resistance due to unusual rapidity of spread locally might well be coincident with one largely attributable to length of exposure but would hardly be expected to overshadow the latter unless associated differences in time of exposure were moderate or slight, a contingency that seems on the whole improbable.

Among solanaceous plants outside the genus *Nicotiana* there are some cultivated species that are susceptible and others that are resistant in one way or another to tobacco-mosaic disease (8). Their distributions accord well with what has been deduced already from a consideration of *Nicotiana* species. Among the susceptible species are the petunia, *Petunia hybrida* Vilm., derived from Argentinian species (1), and the garden huckleberry, *Solanum nigrum* L., probably of European origin. All varieties of these two cultigens seem highly susceptible. Belladonna, *Atropa belladonna* L., native in Europe and Asia, and henbane, *Hyoscyamus niger* L., of Mediterranean origin (1), grown as sources of alkaloids, are likewise susceptible. Highly resistant, hypersensitive species are the Jerusalem cherry, *Solanum capsicastrum* Link, of Brazil, and the Irish potato, *S. tuberosum* L., of Bolivian-Peruvian origin (12). In the genus *Browallia*, one species, *B. speciosa* Hook., of Colombia, is known in two morphologically identical types, one hypersensitive and thus resistant, the other susceptible to chlorotic mottling (5). Another, *B. americana* L., of Peru, Ecuador, Colombia, Venezuela, and Brazil, completely suppresses systemic movement of virus and would thus resist spread of disease from plant to plant in the field.

The common eggplant, *Solanum melongena* L., presents a more complex problem. It is believed to have had an Asiatic origin (1). Its varieties that are still grown in Asia are not known to show a hypersensitive response; instead, insofar as tested, they show chlorotic mottling or become infected without obvious symptoms after experimental inoculation (3, 8). This finding is in agreement with what is known of other solanaceous crop plants of Old-World origin. The eggplant as known in the New World, however, differs greatly from Asiatic types in color, shape, and response to infection;

such a variety as the popular Black Beauty, for example, displays a dark brown pigmentation in addition to the bluish or purplish color common among Asiatic types, a broadened ovate shape, and a hypersensitive response that prevents spread of tobacco-mosaic virus from plant to plant in the field. This effective resistance of one type of eggplant is not in accord with what is known of other solanaceous crop plants of Old-World origin, but its occurrence only in New-World representatives of the species would be understandable if hybridization with some hypersensitive species of *Solanum* had occurred in the Western Hemisphere and remained unrecognized.

Most varieties of the pepper, *Capsicum frutescens* L., are susceptible but one is notably hypersensitive and resistant to disease in the field. This exception is the Tabasco pepper (3), which does not cross readily with most other peppers and may therefore represent a distinct species. As its name implies, this resistant pepper was first obtained from the State of Tabasco in Mexico. Most other peppers are said to have been derived from South American sources. The so-called Red Chili pepper, which hybridizes freely with many other peppers, is fully susceptible. Because of its name, it is presumed to have come from Chile, in the area characterized on the whole by susceptible species of *Nicotiana*. Two varieties of intermediate character, with moderately effective resistance based on slight hypersensitivity, are Long Red Cayenne and Sunnybrook Cheese (4). The original home of these types is not known, though the name Long Red Cayenne suggests origin of one of them at least in northern South America, near Cayenne, capital of French Guiana. Such an origin would accord well with what is known of the distribution of other resistant plants in generally susceptible groups.

DISCUSSION

The three crop plants that have been most adversely affected by tobacco-mosaic disease, namely tobacco, garden pepper, and tomato, are of New-World origin. Though in a general way these three are susceptible as species, they would be expected on theoretical grounds to have resistant allies not too distantly related for use in hybridization. As a matter of fact, in each case effective sources of resistance have been found already and their geographic origins have accorded well with expectation.

For tobacco, resistance depending on two recessive genes determining tolerance was found by Nolla (10) in a Colombian race of tobacco known as the variety Ambalema. Another form of resistance, depending on a single dominant gene determining hypersensitivity (6), has been derived from *Nicotiana glutinosa*, of Peruvian origin.

For the garden pepper a highly effective resistance depending on a single dominant gene for hypersensitivity was found in the Mexican variety that is known as the Tabasco pepper (3). Resistance depending on a less effective and imperfectly dominant allele of the same gene has been found

in two varieties, known respectively as the Long Red Cayenne and Sunnybrook Cheese peppers (4), the former presumably from French Guiana.

For tomato, forms of resistance for which the modes of inheritance have not been fully investigated have been noted as characterizing two closely related wild species, *Lycopersicon hirsutum* Humb. & Bonpl. (11) and *L. chilense* Dun. (7). The first of these is regarded as a native of Peru and the second of northern Chile, near the Peruvian border.

If additional or more effective resistances should be needed for protection of these particular crop plants from tobacco-mosaic disease, suitable resistant relatives might be sought most logically in the areas of resistance indicated in the present paper. On the other hand, if the present world-wide distribution of tobacco-mosaic virus should result eventually in serious damage to crops based on Old-World species, such as belladonna (*Atropa belladonna*) and henbane (*Hyoscyamus niger*), from which the alkaloids atropine and hyoscyamine are obtained, resistance might be much more difficult to obtain, in view of the probable lack of appropriate selection in the past among their relatives and close allies. New techniques might be required to cope with such a situation should it arise.

SUMMARY

Evidence to support the hypothesis that tobacco-mosaic virus was of New-World origin is adduced from a study of solanaceous plants, through a comparison of geographical areas characterized by relatively resistant species and varieties in generally susceptible groups of plants with larger areas characterized by closely allied but highly susceptible species.

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NEW YORK, NEW YORK

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BACTERIA IN HEALTHY POTATO TISSUE[1]

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It was the purpose of this study to determine whether bacteria are normally present in the internal tissue of the potato plant and, if so, to determine the particular tissue or tissues in which they reside, to identify them, ascertain their mode of entry into the plant, and formulate a theory about their occurrence.

The possible presence of microorganisms in healthy tissues of higher plants has been of interest since 1876, when Pasteur (16) reported that grape juice, removed aseptically from the interior of the fruit, remained unaltered. The many papers published on the subject from 1876 to 1896 have been reviewed by Smith (24) and Berthold (4). Microorganisms were obtained in a few cases, but the net effect of these early efforts served only to inculcate the belief that healthy tissues of sound plants are free of microorganisms. From 1896 to 1935 only four papers treated the problem. Since 1935, the majority of the 18 papers published deal with bacteria. Although the authors of these papers report viewing bacteria in healthy tissue or isolating bacteria from internal tissues of higher plants, there are, with few exceptions (11, 12, 15, 20, 21), no logical trends in the results nor are they reducible to rule. A review of the complete literature is in thesis manuscript (12).

Work on the specific problem has had but little impact upon the classic concept that healthy plant tissues are free of microorganisms; however, the concept has been modified as a result of studies in the fields of symbiotic, mycorrhizal, and bacteriorrhizal relations of the higher plants. Primarily responsible for this change of concepts, insofar as bacteria are concerned, have been Perotti and his associates (17), who found bacteria in healthy root tissue of many plants. It is interesting that these "bacteriorrhiza," a term used originally by Hiltner (10) to describe formations of bacteria upon and in the roots of plants, are confined to plant roots and are found only in cortical tissue and not within the vascular system. As now stated, the concept holds that, normally, microorganisms are not systemically distributed in the interior of sound plants.

The author's interest in the problem arose from the discovery that plugs of tissue removed aseptically from the interior of healthy potato tubers and submerged in sterile water frequently yielded cultures of bacteria (27,

[1] Condensed from a manuscript submitted in partial fulfillment of requirements for the degree, Doctor of Philosophy, University of Nebraska, 1949, and based on investigations conducted at the Nebraska and Connecticut Agricultural Experiment Stations. Published with the approval of the Director of the Connecticut Agricultural Experiment Station.

[2] The writer expresses his sincere appreciation to Dr. R. W. Goss for guidance throughout the course of the work. Thanks are due Dr. N. R. Smith for identification of two bacterial species.

28). The present work is therefore a sequel to this earlier research and is confined to bacteria-potato plant relations.

METHODS OF ISOLATION

In preliminary experiments to increase the frequency of positive isolations from internal potato tissue, 50 tubers each of several lots of potatoes[3] were halved; one half of each tuber was isolated from immediately, as described below, and the other half was planted in peat; after a 2-week interval, isolations were made from the seed piece[4] on the young plants. Only from the planted seed pieces were appreciable numbers of bacterial cultures obtained, but a small percentage of cultures resulted, in the immediate isolations from cut tuber halves. It was therefore apparent that much of the work should be done with a single clonal lot of potatoes, to allow a rational comparison of bacterial types and numbers from the different organs.

A single lot of Nebraska certified Bliss Triumph potatoes[5] was used for the bulk of seed-piece, aerial stem, and tuber isolations. Seed pieces cut from this lot were thoroughly suberized, treated with ethylene chlorohydrin, and planted in three different substrates at a daily temperature range of 55°–65° F. to induce sprouting.

Isolations were made from aerial stems and seed pieces of young plants 2–6 weeks old, growing in the greenhouse in peat and composted soil. The plants were dug carefully to avoid injury and washed in tap water. In the laboratory the entire plant was placed in 1:500 acidulated mercuric chloride for 5 min., blotted to remove excess moisture, and then transferred to a small isolation chamber. The stem epidermis was stripped down with a sterile knife, and a wedge-shaped piece of tissue approximating 5×10 mm. outside dimensions was removed from the exposed stelar region with a sterilized scalpel. Tissue pieces were removed from the aerial stem 4–8 in. above the seed piece. The sampling of both seed pieces and tubers involved partial cutting and then breaking the organs into halves. Scalpels, one for each region of the organ to be sampled, were removed from alcohol and flamed. Two pieces of tissue, each measuring approximately $5 \times 5 \times 10$ mm. and together weighing 0.5 gm., were excised from the broken surface of each of four tuber regions (Fig. 1). Region A is comprised principally of cortical tissue but frequently contains outer phloem in parenchyma, rarely xylem tissue of the vascular ring and central pith. Region B contains always the xylem tissue of the vascular ring and both outer and inner phloem in parenchyma. Some cortical tissue is frequently included, and rarely central pith tissue. Region C always contains inner phloem in parenchyma and frequently central pith tissue. Region D includes central

[3] Six lines of potatoes were kindly supplied by Dr. H. O. Werner.

[4] "Seed piece" will be used throughout this paper to mean seed piece of a young plant.

[5] Supplied through the courtesy of Mr. Marx Koehnke of the Nebraska Certified Seed Growers, Alliance, Nebraska.

pith tissue and rarely inner phloem in parenchyma. The degree of vascularization of the different sample region is: A, low; B, high; C, medium; and D, very low. Region D was omitted in isolations from seed pieces because the process of cutting the tuber into seed pieces largely destroyed this portion.

Tissue pieces were transferred directly to liquid media or were macerated by the method of Lutman and Wheeler (15) and transferred to plates of molten nutrient agar.

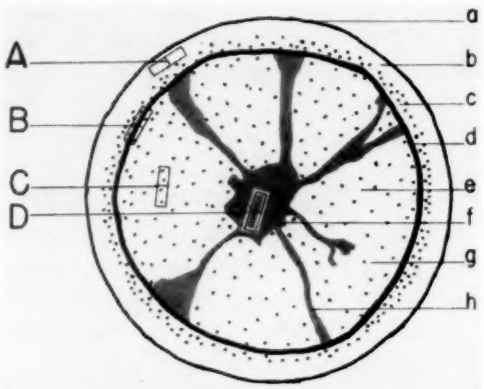

FIG. 1. Diagram of internal cross-sectional anatomy of a potato tuber. A, B, C, and D indicate sampling regions. Samples were duplicate tissue pieces each approximating $5 \times 5 \times 10$ mm. Principal tissues are: a, periderm; b, cortex; c, outer phloem in parenchyma; d, vascular ring containing xylem; e, inner phloem in parenchyma; f, central pith; g, phloem bundle; h, pith strand.

Isolations from potato seeds were attempted by completely macerating surface-sterilized seeds in liquid media and by macerating seedlings reared under sterile conditions on agar slants after flooding of the cultures with sterile water. These two methods were designed to locate bacteria, if present, either within the seed embryo or in tissue external to the embryo. The sequence of steps in seedling isolations were: (a) seed sterilization with an aqueous solution of calcium hypochlorite containing 1 per cent free chlorine; (b) transfer of sterile seeds to upper part of an agar slant and incubation at 28° C. for 72 hr. to test for the presence of bacteria; (c) incubation of seeds at 15° C. to permit germination; (d) reincubation as in b; (e) flooding of seedlings with sterile water, then incubation as in b; and (f) maceration of seedlings with sterile glass rods and incubation at 25° C. for periods up to 14 days to test for presence of bacteria.

All isolation work was done over a damp cloth or inside a specially constructed isolation chamber previously sterilized by liberal atomization with an aqueous solution of propylene glycol.

The medium used primarily for tissue isolations contained, in gm./l.: glucose, 10; peptone, 10; beef extract, 3; KH_2PO_4, 1; KNO_3, 1; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4$, 0.01. Transfers from all cultures in this medium were made to dilution plates of an enrichment modification of Wernham's

buffered potato-dextrose agar containing, in gm./l.: potatoes, 200; glucose, 20; malt extract, 3; yeast extract, 3; $NaNH_4HPO_4$, 0.5; KCl, 0.2; NaH_2PO_4, 0.17; agar, 18. Colonies were picked from the plates and transferred to slants of the same medium for preliminary identification tests.

OCCURRENCE OF CERTAIN BACTERIA IN THE ISOLATIONS FROM BLISS TRIUMPH SEED PIECES, STEMS, AND TUBERS

More than 100 isolates were obtained from the seed pieces and stems of young plants derived from tubers of lot 18. The method of grouping these isolates was as follows: Individual colonies of the different bacteria appearing upon dilution plates prepared from the isolation cultures were transferred to slants and incubated at room temperature. Gram reaction and size measurements were determined for all isolates. Isolates indistinguishable on the basis of these two criteria were then compared as to slant growth characters. Those indistinguishable on the basis of all three criteria were, for purposes of this study, considered the same species. Only one isolate of each species was retained for identification studies after all isolation experiments and grouping of the bacteria had been completed.

Seed-piece isolates obtained more than once from regions B and C were used as standards of reference from other isolates obtained from regions B and C of tubers, from stelar regions of aerial stems, and from seeds and seedlings.

Each of 14 species of bacteria was obtained more than once from regions B and C of seed pieces. The frequency of isolation for the first eight types was: 23, 22, 10, 6, 5, 3, 3, 3. Each of the remaining six types was isolated twice. Nineteen other species, obtained once each from the same seed-piece regions, could not be identified with any of the other isolates from seed pieces. Conversely, still another group of isolates from aerial stems, tubers, seeds, and seedlings could not be identified with any of the bacteria from seed pieces. Among these were several of the Micrococcaceae.

In line with previous reports (9, 11, 21, 28), a considerable number of mixed cultures were isolated. Generally these cultures comprised only two, but sometimes three species. No differential effects due to substrate on either numbers or types of bacteria were noted.

Fourteen bacterial cultures were obtained in 380 attempted isolations from regions B and C of tubers of lot 18. Four of the isolates were identified with isolate 4 from seed pieces of the same lot. Two other isolates were indistinguishable from 1 and 6.

CHARACTERIZATION OF THE BACTERIA

Numbers 12, 13, and 14 of the bacteria from Bliss Triumph seed pieces died in cultures. The remaining 11 bacteria, each represented by one culture, were subjected to 26 determinative tests. *Erwinia carotovora* (Jones) Holland was used as a control organism in all tests. Gram reaction was determined by the method reported by Racicot et al. (18). Other methods

were those of the Manual of Methods of the Society of American Bacteriologists (26). Some of the results are in tables 1 and 2, and names are given in table 2 whenever positive identification could be made.

No isolate produced indole. All grew better on solid and in liquid media at 23° than at 38° C., except 2 and 5, which grew equally well at both temperatures. All were obligate aerobes except 1, which could multiply facultatively as an anaerobe, and 4, which was microaerophilic. All were noncapsulate. Growth characters were studied on agar slants, agar plates, and gelatin plates, and in gelatin stabs. Size and shape data were also recorded.

The only isolates identified with any degree of certainty were 1, 4, 11, and 13. Number 13 died in culture but was retained sufficiently long for a morphologic study. The ability of this isolate to change from a Gram negative short rod to a Gram positive coccus within 48 hr. after original transfer; its growth characters, size, shape, and apparent fastidious nutrient requirements, all indicated that it must be similar to *Bacterium globiforme* Conn (3). Isolates 1 and 4, respectively, were identified by Nathan R. Smith as *Aerobacter cloacae* (Jordan) Bergey et al. and *Bacillus megatherium* De Bary.

The isolates possess certain common attributes: ability to produce alkaline reactions in the presence of simple sugars as well as in plain broth, inability to form gas from carbohydrates, and marked proteolytic action in the presence of gelatin. All are less active physiologically than *E. carotovora*. None of the isolates are encapsulated and none produce indole. A fair proportion of chromogens is present. Lochhead (13) studied bacteria from the rhizospheres of red clover, mangels, oats, tobacco, corn, and flax as compared with bacteria from control soil. His report of higher percentages of forms developing well on nutrient agar, of liquefying types and of those causing acid or alkaline reactions in dextrose, of motile organisms, and of chromogenic types in the rhizosphere might well describe the isolates obtained from healthy seed pieces in the present work.

Nineteen isolates, including 12 from seed pieces and seven from tubers, died on Wernham's enriched potato-dextrose agar. The exacting nutrient requirements of certain rhizosphere-inhabiting bacteria have been recorded (14, 31).

The frequent occurrence of *A. cloacae* in seed pieces is not surprising, since it is widely distributed in nature as a saprophyte and has been isolated from soil, water, and plant surfaces (3). It has been especially abundant on decaying vegetable matter high in pentosans and pectic substances, such as cornstalks and grain (8, 19). Clark, Hervey, and Blank (5) reported its occurrence in the surface soils of the northern cotton-growing areas of Texas. Aerogenic types, including *A. cloacae*, constituted about 0.15 per cent of the total bacterial flora on the root surfaces of cotton and other field and garden plants. These authors also isolated the organism from partially opened, immature cotton bolls. Lutman and Wheeler (15) isolated nu-

TABLE 1.—*Some characteristics of the bacteria from seed pieces of Bliss Triumph potato, lot 18*

Culture No.	Gram reaction	Shape	Spores	Flagella	Potato chromogenesis	Nitrate reduction	H_2S	Gelatine liquefaction
1	−	rod	−	polar	white to gray	+	+	−
2	−	rod	−	−	white to gray	−	+	+
3	−	rod	−	polar	cream	−	+	−
4	±	rod	+	−	cream to tan	+	−	+
5	+	rod	−	polar	white to gray	−	−	+
6	±	rod	−	−	cream to light yellow	−	−	+
7	−	rod	−	−	orange yellow	+	+	+
8	±	rod	−	−	light yellow	−	−	+
9	−	rod	−	polar	gray to brown	+	+	−
10	±	rod	−	−	bright yellow	−	−	+
11	±	rod	+	−	white to gray	−	−	+
12	−	rod	−	.....	.....	.....	.....	.....
13	−+	rod coccus	−	.....	.....	.....	.....	.....
14	−	rod	−	.....	.....	.....	.....	.....
E. carotovora control	−	rod	−	peritrichic	white to gray	+	+	+

..... indicates no test; ± indicates Gram variable; −+ indicates Gram minus, then Gram plus.

TABLE 2.—*Additional characteristics and designation of the bacteria from seed pieces of Bliss Triumph potato, lot 18*

Number of culture	Milk reaction	Plain broth reaction	Glucose		Sucrose		Lactose		Starch	
			R	G	R	G	R	G	R	G
1[a]	Acid red.	Alk.	Acid	+	Alk.	−	Acid	−	Alk.	+
2	Alk. red.	none	Acid	−	Acid	−	none	−	do	−
3	do	Alk.	Alk.	−	Alk.	−	Alk.	−	do	−
4	do	none	Acid	−	Acid	−	Acid	−	Acid	−
5	do	Alk.	Acid	−	Acid	−	Alk.	−	Acid	−
6	do	none	Acid	−	Acid	−	do	−	Alk.	−
7	do	Alk.	Acid	−	Acid	−	do	−	Acid	−
8	Alk.	do	Acid	−	Acid	−	do	−	Acid	−
9	Alk. red.	do	Alk.	−	Alk.	−	do	−	Alk.	−
10	do	do	do	−	none	−	do	−	do	−
11	do	none	none	−	Acid	−	Acid	−	Acid	−
12	.....[b]	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
E. carotovora control	Acid red.	Alk.	Acid	+	Acid	+	Acid	+	+	−

[a] Identity of cultures: (1) *Aerobacter cloacae* (Jordan) Bergey et al., (4) *Bacillus megatherium* De Bary, (11) *Bacillus* sp. Cohn, (13) *Bacterium globiforme* Conn., (14) *Erwinia carotovora* (Jones) Holland.

[b] ..... indicates no test.

merous colonies of *B. megatherium* from healthy potato tubers, particularly of the Burbank Russet variety.

PREVALENCE AND LOCATION OF BACTERIA IN SEED PIECES AND TUBERS

Seed pieces. It was found early in the work that relatively high percentages of positive isolations could be obtained from seed pieces of young plants grown in a peaty substrate in the greenhouse. Six experiments were made to obtain further information on the prevalence and location of bacteria in seed pieces of potato lot 18. Isolations were made from both seed pieces and aerial stems of the young plants in three of the experiments.

TABLE 3.—*Prevalence and location of bacteria in seed pieces of Bliss Triumph potato, lot 18, based on tissue isolations in liquid media*

Substrate for sprouting	Total seed pieces and tissue isolations per region[a]	Bacterial cultures per region		
		A	B	C
Previously used peat	40	3	7	1
Do	20		12	4
Do	40		12	
Do	38		7	5
Previously unused peat	38		8	5
Composted soil	40	3	9	4
Percentage of positive isolations		7.5	25.5	10.8

[a] Duplicate tissue pieces each approximating $5 \times 5 \times 10$ mm. were transferred to tubes of liquid media.

The results (Table 3) indicate: (a) The prevalence of bacteria in the seed pieces is proportional to the degree of vascularization of the tissue. (b) Bacteria do not penetrate the seed piece directly. (c) Bacteria must be generally distributed throughout all vascularized regions of the seed piece. (d) Bacteria may be located within the vascular elements of the seed piece.

Isolations from the stelar tissue of aerial stems yielded percentages of bacterial cultures comparable with those from region B of seed pieces.

Tubers. More than 1,500 tissue isolations made from the four regions of tubers of lot 18, under a variety of cultural modifications, failed to reveal consistent differences in numbers of bacteria among the different regions. In one experiment involving 600 isolations, percentages of bacterial cultures from the four regions were: A, 5.3; B, 4.7; C, 2.7; D, 5.3. The total of positive isolations for all four regions in another experiment was 13 in 560, or 2.3 per cent. Bacteria are more numerous in seed pieces than in tubers.

Although the method of placing pieces of tissue in tubes of liquid nutrient was sufficiently sensitive for the consistent isolation and location of bacteria in seed pieces, for the tuber isolations, the method of Lutman and Wheeler was employed in combination with methods reported above for sampling different tuber regions. Small Irish Cobbler tubers of a lot designated as No. 19 were used, 5 months after harvesting. These ranged

from 2.5 to 3.5 in. in diameter. Tuber regions sampled were: B, as previously defined; C, including inner phloem in parenchyma adjacent to the vascular ring; and C′, inner phloem in parenchyma adjacent to the central pith.

The results (Table 4) support those obtained previously with seed pieces

TABLE 4.—*The prevalence and location of Bacillus megatherium De Bary in Irish Cobbler potato tubers, lot 19, based upon isolation from macerated tissue in nutrient agar*

Number of tubers[a]		Number of colonies per region		
Sampled	Yielding *B. megatherium*	B	C	C′
8	2	1	1	
8	5	15	4	
5	2	6	0	2
5	3	9	0	1
5	4	6	2	2
5	4	8	2	1
5	0	0	0	0
41	20	45	9	

[a] Duplicate tissue pieces approximating $5 \times 5 \times 10$ mm. were macerated and transferred to single Petri dishes for each tuber and region sampled. A total of 41,000 cu. mm. of tissue from regions B and C yielded 54 colonies of *B. megatherium*.

of lot 18 insofar as location of the bacteria is concerned. Colonies representing types other than *B. megatherium* comprised less than 20 per cent of the number isolated. Furthermore, 90 per cent of the plates contained pure cultures of a colony type resembling *B. megatherium*. The other types present developed chiefly as small colonies difficult to detect. Two of the isolates were identified by Nathan R. Smith as strains of *B. megatherium*.

Region A might have been included in the isolations in order to escape the criticism that these results represent merely a decrease in bacterial numbers with distance from the tuber surface. If this were true, region C should yield greater numbers than region C′. A glance at table 4 shows that this did not occur. The close agreement between these results and those shown previously for sprouted seed pieces hardly appears to be fortuitous.

Similar results were obtained in isolations from seed pieces of lot 19 planted in field soil and sampled after 6 weeks in the same manner as the tubers. Eight seed pieces yielded 239 colonies from region B and 32 from region C. Numbers of bacteria were much greater from the seed pieces than from tubers. Eight thousand cu. mm. of tissue from regions B and C yielded 271 colonies.

The most prevalent type of bacterium isolated from the seed pieces on young Irish Cobbler plants was an aerobic sporeformer morphologically indistinguishable from *B. megatherium*. This fact, coupled with previous results, indicates that this organism may well be the most consistent inhabitant of the potato plant grown in natural field soil. It must be em-

phasized that this organism was not the only one present in these seed pieces and that the isolates constituted a mixed flora.

Relative numbers of bacteria in seed pieces and tubers. The foregoing data are comparable and indicate, on the basis of colony numbers, that bacteria were much more numerous in seed pieces than in tubers of the same clonal lot. Bacteria in the seed pieces of lot 19 were 25 times as numerous as in the tubers; one-fifth as much seed-piece tissue yielded 5 times as many bacterial colonies as the tuber tissue. This explains the difference in numbers of bacterial cultures from seed pieces of lot 18 as compared with tubers of the same lot, and the failure to determine the principal location of bacteria in tubers by removal of pieces of unmacerated tissue to tubes of liquid media.

Relative numbers of bacteria in xylem and phloem tissues of tubers and seed pieces. The prevalence of bacteria in tubers and seed pieces is proportional to the degree of vascularization of the tissue. Tissue vascularization here refers to the permeation of parenchymal tissue by principal conductive elements such as vessels and sieve tubes. The degree of vascularization of potato tuber tissue is a function of the relative abundance of the vascular elements ramifying through storage parenchyma. Since the xylem is restricted to the narrow vascular ring, whereas phloem bundles are dispersed to a much greater degree by parenchyma, tissue containing both xylem and phloem is more heavily vascularized than tissue containing phloem only.

The gradient of bacterial numbers correlates with anatomical differences in the tuber regions sampled, but it furnishes no information on the relative numbers of bacteria in the conductive elements of the xylem and phloem.

Vessels, sieve tubes, and conductive parenchyma in the potato tuber are of sufficient diameter to accommodate bacteria (2, 6). Photomicrographs (2, 7) show that the pits and pores of vessels and sieve tubes are probably of sufficient diameter to permit passage of bacteria. The exceedingly small numbers of bacteria from the phloem in parenchyma of region C suggest that only one of the cell types in this tissue complex may harbor bacteria. Logically this would be the sieve tube. It will be assumed for the purpose of analysis that bacteria are located only in vessels and sieve tubes of the respective tissues.

The problem was to derive theoretical ratios of the number of bacteria in xylem to the number occurring in phloem in parenchyma, and to the number occurring in phloem tissue of the tubers, based upon data in table 4 and supplementary data summarized in table 5. The relative volumes of xylem and outer and inner phloem in parenchyma tissues were calculated from actual measurements of total and vascular ring diameters made on 20 tubers of lot 19. In making the measurements the outer phloem was considered to permeate all tissue outside the vascular ring and the inner phloem to permeate all tissue inside the vascular ring. Width of the vascular ring was considered to be 1 mm. Samples of tissue removed from regions B and

C approximated 500 mm.^{3} volume. The relative proportions of the three tissues in samples from regions B and C are in table 5.

TABLE 5.—*Theoretical ratios of the number of bacteria in different tissues of the potato tuber, based upon actual numbers from regions B and C of Irish Cobbler tubers, lot 19*

Tissue[a]	Relative volume in tuber[b]	Volume of sample, mm.^{3}		Distribution			
				Based on actual numbers		Theoretical, based on relative tissue volumes	
		B	C	B	C	B	C
Outer phloem in parenchyma	0.401	200	0	3.6	0	1.4	0.0
Xylem	0.036	100	0	37.8	0	1.4	0.0
Inner phloem in parenchyma	0.563	200	500	3.6	9	2.0	5.1
Total	1.000	500	500	45.0	9	4.8	5.1
Sample ratio				5.0	1		
Xylem-phloem in parenchyma ratio				21.0	1		
Theoretical xylem-phloem ratio						1.5	1.0

[a] Outer phloem in parenchyma means outer phloem embedded in parenchyma; xylem is xylem of the vascular ring; inner phloem in parenchyma means inner phloem embedded in parenchyma.

[b] Assumed to be a measure of dispersion of conductive tissue in parenchymal tissue.

The preponderance of bacteria in tissue pieces from region B as compared with the numbers in an equal volume of tissue from region C must be due to the presence of xylem. Since 500 mm.^{3} of inner phloem in parenchyma from each of the tubers sampled yielded 9 colonies of *B. megatherium*, it is logical to assume that 200 mm.^{3} of inner and outer phloem embedded in parenchyma in the tissue pieces from region B each accounted for 3.6 colonies of *B. megatherium*. The remainder of the colonies from region B must have come from the vascular ring containing a large fraction of xylem tissue. A comparison of numbers from equal volumes of xylem and from phloem embedded in parenchyma yields a ratio of 21 to 1. This ratio (Table 5) is derived from the 5 to 1 ratio actually obtained from samples of regions B and C (Table 4).

The 21 to 1 ratio reveals nothing of the relative numbers in different regions of the tuber on a cellular basis. Bacteria are probably located only in vessels and sieve tubes of the respective tissues.

The relative volumes of the three vascularized regions, viz., xylem and outer and inner phloem in parenchyma, apparently constitute a measure of the concentration or dilution of elements containing bacteria. To correct for the dispersion of phloem elements in parenchyma, the number of bacteria in each tissue, based on isolations, was multiplied by the relative volume of that particular tissue in the tuber. For example: $0.401 \times 3.6 = 1.4$ colonies from outer phloem tissue of region B (Table 5). The calculation

of these values is followed by the same procedure which developed the 21 to 1 ratio. There are 1.4 colonies of *B. megatherium* from 100 mm.^{3} xylem to compare with numbers from an equal volume of phloem. Thus: $5.1/5 = 1.02$ colonies in 100 mm.^{3} inner phloem. Averaging numbers in both outer and inner phloem yields a slightly lower value: $(1.4 + 2.0)/4 = 0.85$ colony of *B. megatherium* from 100 mm.^{3} of phloem tissue.

On the basis of relative volumes, if it were possible to isolate bacteria from xylem tissue only and to compare the numbers with those obtained from the same volume of equally concentrated phloem, then the sample ratio should be about 1.5 to 1 (Table 5). This theoretical ratio does not differ widely from unity and indicates that bacteria may exist in both types of vascular elements in approximately equal numbers.

This analysis is based on an assumption that both vessels and sieve tubes are of equal volume in the tuber and that the phloem is dispersed relatively to the xylem in proportion to the volumes of tissue which they occupy in the tuber. Actually there is good evidence that the phloem exceeds the xylem in the tuber (2). If this is true, then the use of relative volumes results in an over-compensation for phloem dispersal. This fact, coupled with the results of the analysis, indicates that bacteria are slightly more numerous in xylem than in phloem conductive elements.

The location of bacteria in seed pieces and tubers and their relatively small numbers do not support the theory proposed by Lutman and Wheeler, that the denser material between plant cell walls is not calcium pectate as reported by Mangin, but living microorganisms that may be grown in culture media outside the plant.

SEED AND SEEDLING ISOLATIONS

In view of the probable discontinuity of the vascular traces in the outer ovular integument (1, 7), the finding of bacteria in the inner tissues of the embryo or young seedling reared under sterile conditions would constitute a departure from the results of tuber and seed piece isolations. Further, Schanderl's contention (23) that bacteria are present intracellularly in the parenchymal tissue of certain *Galium* spp. would command serious attention.

The purpose of this part of the study was to ascertain whether bacteria, if they occur commonly in potato seed, are in the embryonic tissues or in the seed integuments.

More than 450 isolations were attempted from seeds and seedlings. Although bacteria were obtained, their occurrence was not common (Table 6). Further, there was no marked difference in the percentages of bacterial cultures in seed or seedling isolations, and none of the isolates could be identified with bacteria from seed pieces.

When these results are compared with those from maceration experiments performed with potato tubers, where less than 1 per cent of the total tissue volume was removed for sampling, it must be inferred that they do

TABLE 6.—*Number of potato seeds and submerged seedlings yielding bacteria, in terms of number tested*

Seed family	Seed cultures in liquid media		Submerged seedlings on agar slants	
	Macerated	Unmacerated	Macerated	Unmacerated
127.43—1 × Katahdin[a]	1/40	0/28	3/39	0/20
117.43—3 × Pawnee	0/40	0/20	1/40	0/20
127.43—1 × 120.46–6	1/28	1/8		
75–5 (1935)[b]	1/20	1/7		
75–5 (1936)	0/20	0/20		
75–5 (1937)	2/20	1/9	1/21	1/19
75–5 (1938)			1/40	

[a] Hybrid seeds supplied by H. O. Werner, University of Nebraska.
[b] Seeds from lines inbred from year indicated to 1947 supplied by F. A. Krantz, University of Minnesota.

not indicate any common or systemic occurrence of bacteria in potato seed or seedling tissues.

TESTING OF THE ROOT-ENTRY HYPOTHESIS

The foregoing data indicate that bacteria may enter the plant via the root system. A root-entry hypothesis is supported indirectly by: (a) the preponderant numbers of bacteria in region B of seed pieces and tubers, (b) the greater numbers of bacteria in seed pieces as compared with tubers, and (c) the fact that the majority of bacterial types isolated from seed pieces of young plants grown in enriched substrates displayed physiological characters similar to those of bacteria isolated from the rhizosphere of certain plants.

The mode of entry through the roots has been postulated as occurring at the point of emergence of secondary roots (28). Actually pericyclic and parenchymal tissues impose cell barriers between bacteria at the point of origin of secondary roots and the vascular elements. Furthermore, the vascular system is closed and the entrance of bacteria would be contingent upon a rupture in the vascular elements as well as in pericyclic and parenchymal cells.

An alternative explanation[6] as to the mode of entry into roots is the view that bacteria might penetrate directly into undifferentiated meristematic root tissue. Bacteria residing in those cells later differentiated into vascular elements would be in a more favorable position for transport to other parts of the plant than those present in cells later differentiated into parenchymal tissue. This explanation does not conflict with the present results and it may be conceded that direct penetration through undifferentiated meristematic cells is perhaps more likely than penetration through pericyclic, parenchymal, and vascular tissue.

Only one aspect of the root-entry hypothesis has been tested in the pres-

[6] The author is indebted to Dr. H. L. Weaver, University of Nebraska, for this viewpoint.

ent work, viz., do bacteria enter the plant through the root system? Several inoculation and reisolation experiments with bacteria were made, to test the hypothesis directly. Two requirements are imposed upon the design of such experiments. First, the species of bacterium selected must be unique in order to facilitate identification, and second, it must not be a normal inhabitant of the potato plant. A pathogenic form which can be readily identified by transfer to an appropriate host plant meets both conditions. The normal occurrence of pathogens in the healthy tissue of nonsuscepts has not been established, although Valleau, Johnson, and Diachun (30) reported the presence of pathogens on root surfaces.

Two pathogens were selected: *Pseudomonas tabaci* (Wolf & Foster) Stevens, and *Ps. phaseolicola* var. *medicaginis* (Burkholder) Dowson. A preliminary test was made to determine 1) whether bacteria injected into stems of potato seedlings could be reisolated at a later date, and 2) the extent of movement from the point of inoculation.

The results of inoculating 20 potato seedlings with *Ps. tabaci* and removal of stem sections from various positions after 30 days demonstrated survival of the organism in all stems at the point of inoculation, and some movement up and down the stems from the point of inoculation. It was concluded that this method could be used effectively for testing the root-entry hypothesis.

In three experiments, potato and tomato seedlings were subjected to heavy suspensions of the organisms poured into sand around their roots. After an appropriate period, tissue pieces were removed from the upper parts of the stems of these plants, transferred aseptically to sterile water blanks, and macerated with heavy glass rods. Leaves of the host plant, tobacco or bean, were water-soaked by means of hypodermic injections along the midveins. The macerant was poured over this water-soaked surface, and numerous pin punctures were made with a sterile dissecting needle. The plants were then incubated at temperatures favorable for the expression of halo symptoms.

In the first experiment utilizing potato seedlings and *Ps. tabaci*, three of ten tobacco seedlings had positive wildfire symptoms. When the experiment was repeated, two of 20 seedlings had positive wildfire symptoms 14 days after transfer to tobacco. Noninoculated controls yielded negative results in both experiments. Fifty tomato plants tested with *Ps. phaseolicola* var. *medicaginis* yielded negative results when macerated stem sections in sterile water were used to inoculate beans. The results of the first two experiments afford some direct evidence in support of a root-entry hypothesis.

DISCUSSION

The normal occurrence of bacteria in the potato plant is indicated by the high percentages of bacterial cultures and colonies from aerial stems, seed pieces, and tubers (20, 21, 15, 11) and by the consistent isolation of

certain bacterial species as constituents of a mixed flora (20, 21, 11). The presence of bacteria is proved by the discovery that their prevalence in seed pieces and tubers is proportional to the degree of vascularization of the tissue (11).

Evidence for the location of bacteria in vascular elements of the potato plant rests, primarily, on isolations from tubers and seed pieces. Theories relating bacteria positionally and functionally to the middle lamella and to their localization in xylem tissue near the stem end of large potato tubers are not supported (15, 21, 23).

Evidence that bacteria are in the vascular systems of healthy potato plants is accompanied by evidence of their absence from embryos of seeds. There is no direct connection between the vascular elements supplying the flower and ovule and those of the embryo (1, 7). If bacteria occurred commonly in seedlings, the contention of Schanderl (22) and of Hennig and Villforth (9), that bacteria occur in every cell of the plant, would command serious attention. On the other hand, the presence of bacteria in vascular elements of the plant and their absence from the embryo can be explained adequately in the light of present knowledge.

The only direct proof of bacterial entry into the plant through the root system is furnished by the reisolation of *Ps. tabaci* from potato seedlings. Tyner (29) concluded that the ring-rot organism could enter the potato plant through the roots. Since both the ring-rot organism and the bacteria obtained in these experiments are inhabitants of the vascular system, they may enter the plant in the same manner.

The bacteria isolated in these experiments were most prevalent in the heavily vascularized tissue removed from region B of seed pieces on young plants. Therefore, it is likely that they do not enter the tuber and seed piece directly through peridermal or epidermal layers. Entry through these layers could occur at the points of rupture caused by root emergence, but this should result in vascular invasion. Location of the bacteria in vascular elements suggests that they may enter the plant via the root system.

The greater numbers of bacteria in seed pieces as compared with tubers of the same potato lot support the root-entry hypothesis. This difference is explained by postulating a progressive dilution of the bacterial content of the conducting substances with increase in distance from the point of entry of bacteria into the plant. The seed piece, being located at the base of the stem, is much nearer the main root system than the tubers, which are on the ends of horizontal branch stems or stolons.

Further support for the root-entry hypothesis is afforded by the types of bacteria obtained. The majority of species isolated resemble types commonly obtained from the rhizosphere of certain plants and possess common physiological attributes.

Ability of bacteria to inhabit the rhizosphere is the first requisite for entry into the plant. The primary screening action of the plant is generic

in scope and is probably exerted outside the plant body by virtue of root excretions. Further discussion on this point must be deferred, inasmuch as the seasonal history of the occurrence of bacteria in healthy potato plants has not been elucidated for plants grown in natural field soils; nor do we possess information concerning types of bacteria which may normally inhabit the rhizosphere of potato plants.

An outstanding feature of the present results is the consistent isolation of *B. megatherium* from seed pieces and from tubers of both the Bliss Triumph and Irish Cobbler varieties. Lutman and Wheeler (15) first reported isolation of this organism in large numbers from Burbank Russet tubers which had been in storage for at least 4 months and had broken dormancy. They also isolated the organism from small freshly harvested Bliss Triumph tubers and from potato roots, sweet potatoes, and carrots. In the present study, *B. megatherium* was isolated from seed pieces and aerial stems as well as from Bliss Triumph tubers less than 2 months after they were harvested, and from Irish Cobbler tubers which had been in low-temperature storage for 5 months.

Present knowledge of the occurrence of *B. megatherium*, therefore, indicates that it is a common inhabitant of the potato plant, and that it can survive in the tuber for as long as 5 months after harvest. Whether this organism is unique in its ability to inhabit the plant is still a matter for speculation. The conspicuous absence of related forms such as *Bacillus cereus* Frankland & Frankland, which is known to outnumber any other member of the genus found in soil (25), suggests that the occurrence and survival of *B. megatherium* in the potato plant may be dependent upon characteristics other than its ability to form spores.

The number of bacterial species isolated, their small numbers, their location in the plant, and their mixed occurrence in the tissue indicate that the bacteria-plant relation possesses certain elements of fortuity.

SUMMARY

Small numbers of many types of aerobic, heterotrophic bacteria were isolated from seed pieces and aerial stems of potato plants growing in peat and composted soil.

The prevalence of bacteria in seed pieces and tubers was proportional to the degree of vascularization of the tissue.

Bacteria were more numerous in seed pieces than in tubers of the same potato lot.

Bacteria could not be isolated consistently from surface-sterilized potato seeds or from seedlings grown under sterile conditions.

Preliminary results indicate that bacteria can enter the healthy potato plant via the root system.

Aerobacter cloacae (Jordan) Bergey et al. was isolated most frequently from seed pieces and aerial stems of plants growing in peat and composted soil.

Bacillus megatherium De Bary and two other species occurred commonly in seed pieces and aerial stems of potato plants grown in peat and composted soil.

Bacillus megatherium De Bary was isolated most frequently from tubers and was the most prevalent organism common to all parts of the potato plant investigated.

The majority of bacterial types possessed physiological characters similar to those reported for bacteria which have been isolated from the rhizosphere of certain plants.

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TREATMENT OF CROP SEEDS IN BRAZIL AND PARAGUAY

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Treatment of crop seeds with fungicides prior to planting has received relatively little attention in Latin America, insofar as published data are concerned. Fischer[2] reported on some experiments with wheat seed treatment in 1938, and a preliminary report[3] which includes seed treatment experiments with a number of crops in Paraguay was published in 1948. A recent mimeographed report[4] gives the results of tests made in Brazil in the State of Rio Grande do Sul. Significantly higher percentages of germination were obtained from seed treated with certain fungicides than from untreated seed of beet, broccoli, cabbage, lettuce, mustard, peas, potato, spinach, squash, tomato, turnip, watermelon, corn, flax, rice, soybean, and wheat.

From September 1947 to November 1949 a number of experiments were made by the writer in Brazil. Because of the limited distribution of the Paraguayan report, as well as lack of statistical analyses of the data, it has seemed desirable to bring the data from the two countries together in one publication for comparison. This present paper deals principally with the effect of fungicides on seed germination, but in some cases yields were obtained. The crops studied are corn, cereals (barley, rice, rye, and wheat), peanuts, sorghum, beans, beets, and peas. The data were obtained between July 1946 and November 1949 and are presented by crops.

Corn. The chemical dusts used to treat corn included Arasan, Spergon, Semesan Jr., Phygon, and Granosan, each at the rate of 2 gm. per kg. of seed. The Granosan was first prepared by diluting the commercial product (5 per cent ethyl mercury phosphate) with talc to make a 1 per cent dust. The method of planting was usually a Latin-square design with as many replications as there were treatments or varieties of seed. With one exception, 100 seeds were planted per 5-meter row for each treatment or replicate. Most of the lots of seed were flint or semi-flint, although three lots of dent corn were included in the several experiments. No yield data were obtained, only the percentage germination by count.

In March 1947 a sample of Venezuela yellow corn (flint) harvested in

[1] The data from Paraguay were obtained while the writer served as Agricultural Officer for the Food Supply Division, Institute of Inter-American Affairs in Asuncion, Paraguay. The data from Brazil were obtained while he served with the American International Association in São Paulo, Brazil.

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[4] Oliveira, Manoel Alves de. Tratamentos de Sementes; sua influencia em cultivos sob condicoes de Campo. A thesis presented to the Department of Phytopathology and Microbiology in the School of Agronomy, Pelotas, Rio Grande do Sul. October, 1949. (Mimeographed.)

1946 at the Instituto Agronômico Nacional in Paraguay was selected and divided into four lots. One was retained as a check and the other three were treated respectively with Arasan, Spergon, and Semesan Jr. Plantings were made on April 10 and May 12. Subsequent plantings were made June 10, July 10, and August 12 with seed of the 1947 crop harvested in May. The differences were negligible except for the last two planting dates, when seed treated with Arasan and Spergon gave significantly higher percentages of germination than untreated seed. The mean percentages for the two planting dates were 83, 92, 93.2, and 81.7 for untreated, Arasan, Spergon, and Semesan Jr., respectively. The minimum difference for significance at the 1 per cent point did not exceed 6.75.

Higher yields of corn are obtained in Paraguay by winter planting (July and August), partly because the corn escapes injury by stalk-borers and earworms. Planting in deep furrows is necessary to insure adequate moisture for germination, and since soil temperature is lower in winter than in the spring months, it may be a valuable practice in the future to treat seed corn for winter planting.

Experiments with seed treatment in Brazil were made in 1948 and 1949. In 1948, seed of a double-cross, semi-flint hybrid was treated with Arasan and Granosan and planted July 1 in peat soil at São José dos Campos in the Paraiba valley in the State of São Paulo. There were five replications of each lot and the mean percentages of germination were 75.4, 80.6, and 84.2 respectively for untreated, Granosan, and Arasan. Analysis by the T-test showed the odds were 99 : 1 that the differences between untreated and Arasan-treated seed were not due to chance. This planting was made in winter in soil high in moisture content and with a lower temperature than in the spring months.

In a second experiment in Brazil in 1948, five varieties, five treatments, and five replications were used at two locations in the State of São Paulo. The planting dates were November 10 and November 18. Two of the varieties were dent, two flint, and one semi-flint corn. The mean percentages of germination by treatment for all varieties in plot 1 and plot 2 respectively were: untreated, 73.9 and 80.5; Arasan, 78.5 and 81.8; Granosan, 76.5 and 83.1; Phygon, 75.9 and 80.7; Spergon, 78.6 and 83.6.

These differences are not significant; this may be explained by the fact that soil temperatures in November in that area are exceptionally favorable for corn germination, and the viability of the seed was high.

In June 1949 a planting was made in the Paraiba Valley at São José dos Campos with seed of five varieties similar to those used in 1948. Only Arasan was used as the treatment and five replications were planted. The mean percentages of germination were 87.7 and 89.3 for untreated and Arasan respectively. The difference was not significant.

These tests in Brazil and Paraguay indicate that only for winter planting of corn may seed treatment be expected to give significant increases in field germination.

Barley and rye. In Paraguay a planting was made in May 1947 with three lots of barley from the United States and a lot of rye produced locally. The treatments used were Arasan, Granosan 1 per cent, and Ceresan 2 per cent, each at the rate of 2 gm. per kg. of seed. Ceresan 5 per cent was also used at the rate of ½ gm. per kg. of seed. There were five replications. The barley varieties were Calhoun, Tenwase, and Nassau. Differences in germination between treated and untreated seed were not significant. The mean percentages by treatment, for three varieties of barley and the one variety of rye respectively, are: Arasan, 67.5 and 82.0; Granosan 1 per cent, 64.5 and 78.3; Ceresan 5 per cent, 63.3 and 80.0; Ceresan 2 per cent, 61.5 and 80.0; untreated, 63.8 and 74.0.

Wheat. A test with five varieties of wheat imported from the United States was planted in May 1947 in Paraguay at the same time as the barley test. The varieties were Carola, Sanford, Purcam, Hardired, and No. 2375. The treatments and rates used were the same as for barley. The mean percentages of germination were 46.3, 47.1, 47.1, 47.1, and 43.1, respectively for Arasan, Granosan 1 per cent, Ceresan 5 per cent, Ceresan 2 per cent, and untreated. The differences were of no significance.

In 1948, seed of five varieties of wheat was obtained from the Federal Experiment Station at Curitiba, Parana, in Brazil. Treatments used are in table 1. A planting was made in June in peat soil at São José dos

TABLE 1.—*Germination of treated and untreated seed of five wheat varieties in Brazil; 1948*

Variety	Percentage germination		
	Arasan	Granosan 1 per cent	Check
Sales	62	36	21
No. 35	56	55	28
Frontana	56	46	26
Kenia	53	45	17
Fronteira	49	45	23
Mean	55.2	43.4	23.0

Campos in the Paraiba Valley using five replications. The differences are striking and were probably due largely to a high percentage of seed infection with the scab fungus, *Fusarium* sp. All lots were equally infected.

In 1949 seed of the wheat varieties Frontana, Sales, and Kenia was obtained from the Federal Experiment Station at Patos in Minas Gerais. Seed of Bandeirantes was obtained from São Paulo. A portion of each lot was treated respectively with Arasan, Granosan 1 per cent, and Seedox, using 2 gm. per kg. of seed. These lots together with untreated seed were planted in May in peat soil at São José dos Campos on land that had produced a crop of rice during the summer months.

The planting was made May 24 in rows 5 m. long, 40 cm. between rows, and with 20 gm. of seed per row. The varieties were arranged in a Latin-square design of four replications and the treatments for each variety were randomized within the replicates. Each replicate consisted of three rows

per treatment. After harvest in October, the wheat in each treatment of three rows by replicates was threshed separately and the weight of seed determined. The data from this test are in table 2. A statistical analysis

TABLE 2.—*Yield from treated and untreated seed of four wheat varieties planted and harvested in Brazil; 1949*

Variety	Arasan	Granosan 1 per cent	Seedox	Check
Frontana				
Gm. per 4 replicates	1,800	2,700	1,900	2,000
Kg. per hectare	599.4	899.1	632.7	666.6
Bu. per acre	8.8	13.2	9.3	9.8
Sales				
Gm. per 4 replicates	6,600	6,300	5,600	4,550
Kg. per hectare	2,197.8	2,097.9	1,864.8	1,481.0
Bu. per acre	32.2	30.8	27.4	21.7
Kenia				
Gm. per 4 replicates	3,470	3,650	3,400	3,050
Kg. per hectare	1,156.5	1,215.5	1,132.2	1,015.6
Bu. per acre	17.0	17.8	16.6	15.0
Bandeirantes				
Gm. per 4 replicates	2,900	2,830	2,830	1,880
Kg. per hectare	965.7	1,098.7	942.4	626.0
Bu. per acre	14.2	16.1	13.8	9.2
Total gm.	14,770	15,480	13,730	11,480

of the data show that, based on total yields for all varieties, the differences are highly significant. The respective totals (yield in gm.) were 14,770 for Arasan, 15,480 for Granosan, 13,730 for Seedox, and 11,480 for the check. The minimum difference for significance at the 1 per cent point is 2,466; hence, the yields from Arasan and Granosan treated seed are significantly higher than from the check but not from Seedox. Also there is no significant difference in yields from Seedox and the check.

A second planting from the same lots as used for the experiment in table 2 was made May 25 using a Planet Junior drill to plant three rows of each lot starting with the variety Frontana treated with Arasan, then Granosan, Seedox, and untreated. The other three varieties were planted in this same order. The rows were 140 m. in length and 40 cm. between rows, and the rate of seeding was 90 kg. per hectare. The soil was level and uniform. Without replications it was impossible to make a statistical analysis, but the increase in yield resulting from treatment with Arasan was outstanding on all varieties. The mean yields in bushels per acre were 10.8, 11.7, 13.8, and 17.1 respectively for untreated, Seedox, Granosan 1 per cent, and Arasan.

Rice. In 1946 a seed treatment test was planted in Paraguay using one variety of rice and four commercial dusts at the same rate of application as given for barley. The mean percentages of germination were 26.6, 30.6, 34.2, 30.4, and 23.4 for untreated, Arasan, Semesan Jr., Ceresan 5 per cent, and Ceresan 2 per cent, respectively. The differences are not significant.

In Brazil a planting was made in November 1949 at São José dos Campos

using a local variety. Four commercial dusts were used at the same rates of application as in the Paraguayan experiment. There were four rows each 5 m. long and 50 cm. between rows per replicate. The soil in the plot was partially decomposed peat; the land was inundated 45 days after planting, and remained so until a week before harvest. The mean yields, in gm. per sq. m., were 1,290, 1,406, 1,300, 1,210, and 1,190 respectively for untreated, Granosan 1 per cent, Phygon, Arasan, and Spergon. The differences are of no significance.

Peanuts. A seed treatment test with peanuts was made in Paraguay in 1946 using seed of five varieties with five replications of 100 seeds per row. Two gm. of dust per kg. of seed was applied. The design was a Latin square with the treatments randomized within each varietal replicate. The data obtained are in table 3; differences in germination between untreated

TABLE 3.—*Percentage germination of treated and untreated seed of five varieties of peanuts at Caacupe, Paraguay, 1946*

Variety	Germination by treatments				
	Untreated	Arasan	Ceresan 2 per cent	Phygon	Spergon
Negrito	69.2	81.0	89.4	84.6	76.8
No. 116	12.2	53.0	60.8	58.8	50.4
Spanish 115	67.2	80.2	78.8	77.6	77.0
No. 106–230	61.6	73.6	81.6	76.4	72.4
No. 109–59	47.6	49.6	52.8	51.6	52.0
Mean	51.6	67.5	72.7	69.8	65.7

The totals for germination of all varieties by treatments are 1289 for untreated, 1687 for Arasan, 1817 for Ceresan, 1652 for Spergon, and 1745 for Phygon. The minimum difference for significance at the 1 per cent point is 113.

and all treatments are highly significant.

Hulless seed of one peanut variety (local) was obtained in Brazil in 1949. Treatments used are in table 4; rate of application was 2 gm. per kg. of seed. Plantings were made in two plots in rows 5 m. long, 50 cm. between rows, five rows per replicate. The design was a Latin square of five replications. Each row was planted with the same weight of seed.

The number of plants and the yield in kilograms per treatment are in table 4. In both plots all treatments gave a significantly higher number of plants than no treatment, but only in plot 2 were the differences in yield significantly greater from treated than from untreated seed.

These results from seed treatment of peanuts are comparable to those obtained in the United States.

Sorghum. Seed of four varieties of sorghum (three imported from the United States) was treated with four different dusts, using 2 gm. per kg. of seed, and planted in Paraguay in 1946. There were five replications of 100 seeds per row. The varieties were Kaffir, Honey Drip, Midland, and Westland. The mean percentages of germination were 47.8, 49.1, 52.2, 48.3, and

TABLE 4.—*Percentage germination and yield from treated and untreated peanut seed in São Paulo, Brazil, 1949*

Treatments	No. of plants		Yield		
	Plot 1	Plot 2	Plot 1	Plot 2[a]	Mean
			Kg.	*Kg.*	*Kg.*
Untreated	406	317	9.0	3.4	6.2
Arasan	673	585	12.1	6.6	9.35
Phygon	764	455	12.8	4.8	8.9
Spergon	741	495	13.4	5.0	9.2
Ceresan 2 per cent	829	571	14.0	5.0	9.5

The minimum difference for significance at the one per cent point in yield (plot 2) is 1.7 kg.

53.0 respectively for untreated, Arasan, Ceresan 2 per cent, Semesan Jr., and Spergon. Taken as a whole the differences were not significant, but for the variety Midland the mean percentage germination of untreated seed was 43.6 and for all the treatments it was 57.3.

In vegetable seed treatment tests in Paraguay, seed of beans, beets, and peas were used. The results are given by crops.

Beans. Seed of five varieties of locally grown seed at the Instituto Agronômico Nacional was selected and treated with four different dusts, 2 gm. per kg. of seed. Four of the lots were Lima beans, the other a field bean. There were five replications of 100 seeds per row and each treatment was randomized within a varietal replicate. The mean percentages of germination were 62.0, 69.0, 64.8, 66.2 and 66.9 respectively for untreated, Arasan, Phygon, Spergon, and Semesan Jr. None of the differences are significant.

Beets. Treatment of seed of the Detroit Dark Red variety, using three different dusts and four replications of each, planted in Paraguay in 1947, gave approximately 100 per cent more plants from treated than from untreated seed. The mean percentages of germination were 12.5, 23.5, 22.5, and 25.5 respectively for untreated, Arasan, Phygon, and Ceresan 5 per cent.

Peas. The treatments with pea seed involved five dusts on 10 varieties. Rates of application were $2\frac{1}{2}$ gm. per kg. of seed. Each of five replicates by varieties contained 100 seeds planted in a row 5 m. long. The treatments were randomized within each varietal replicate. The planting was made April 15, 1947; summarized data are in table 5. The differences are highly significant as between untreated and any one treatment. However, it may be noted that the benefits from seed treatment are much more outstanding for varietal numbers 5, 6, 7, 8, and 10 than for the other five. This result is of some interest because numbers 5, 6, and 7 are selections from Catalana, a smooth-seeded variety, and numbers 8 and 10 are wrinkled-seed types. It is odd that the selections from Catalana responded so much more favorably than the parent variety, but this may be the result of much lower vitality of seed as shown by the selections.

In a second experiment, seed of the variety Catalana was selected and prepared for planting using five treatments and no treatment, each planted

TABLE 5.—*Percentage germination of treated and untreated seed of 10 varieties of peas planted April 15, 1947; Caacupe, Paraguay*

Variety	Germination by treatments					
	Untreated	Arasan	Granosan 1 per cent	Ceresan 2 per cent	Phygon	Spergon
1. Cuarentona	95.4	97.0	99.8	95.4	97.8	98.5
2. Catalana	96.6	96.2	95.2	97.0	95.8	95.0
3. Sel. 1	81.0	84.2	74.6	77.8	81.8	86.0
4. Sel. 3	21.4	21.4	21.6	20.4	23.2	22.4
5. Sel. 4	68.5	85.4	85.8	88.6	91.2	86.8
6. Sel. 6	18.2	26.0	24.2	21.2	26.2	24.6
7. Sel. 7	66.2	78.4	78.0	75.2	81.2	84.6
8. Woodruff Topper	88.4	94.2	96.1	96.0	96.2	96.2
9. Surprise	94.4	93.8	93.8	92.2	95.8	96.8
10. Perfection wilt resistant	84.2	96.0	94.6	94.8	95.0	94.0
Mean	71.4	77.3	76.4	75.9	78.4	78.5

Totals for all varieties by treatments are 3569 for untreated, 3863 for Arasan, 3819 for Granosan, 3797 for Ceresan, 3920 for Phygon, and 3925 for Spergon. The minimum difference for significance at the one per cent point is 98; hence, the untreated is lower than each treatment.

at six different dates (May 12, May 26, June 10, June 26, July 7, and July 25). The planting design for each date was a Latin square of six replications with 100 seeds per row or replicate. The differences were not significant except for two dates of planting, namely May 26 and July 7; the percentages, by treatment, were, for these two dates respectively: no treatment, 81.0 and 84.6; Arasan, 90.0 and 89.6; Granosan 1 per cent, 91.0 and 85.1; Ceresan 2 per cent, 90.0 and 87.0; Phygon, 93.7 and 90.6; Spergon, 86.8 and 91.6.

The totals by treatment for the planting on May 26 (no treatment, 486; Arasan, 540; Granosan 1 per cent, 546; Ceresan 2 per cent, 540; Phygon, 562; Spergon, 521) were highly significant at the 5 per cent point, with a minimum difference of 40 for significance.

The data for the planting on July 7 showed significant differences above no treatment for Arasan, Phygon, and Spergon.

The results for the planting on July 7 also show significance in favor of three of the treatments.

DISCUSSION AND SUMMARY

In experimental treatment of field and vegetable crop seeds with fungicides in Paraguay and Brazil from 1946 to 1949, only dust fungicides were used; with the exception of Seedox, used on wheat in 1949, they were the ones in general use in the United States. Some of the crop varieties had been introduced and found to be adapted, some had been commonly grown in the area for a long time, and some were newly introduced: corn, barley, rice, rye, wheat, peanuts, sorghum, beans, beets, and peas. In general the germinability of the seeds was fairly high, although in a few cases it was medium to low. With one exception, the pathological condition of the seed was not determined in advance of planting because facilities were not avail-

able for laboratory examination. The effect of seed treatment was measured in terms of field germination for most of the tests, although yields were obtained with peanuts and wheat in two experiments. The experiments in Paraguay were conducted at the Instituto Agronômico Nacional, which is 50 kilometers east of Asuncion. Those in Brazil were carried out at several different locations in the State of São Paulo.

The results of these tests may be summarized briefly as follows:

Seed treatment of corn gave significantly higher percentages of germination in Paraguay and Brazil only when the seed was planted in the winter months of June, July, or August when soil temperature is lower than in the spring or summer. The seed used was mostly flint or hard dent. In areas of Brazil farther south where dent corn is preferred, it may be that seed treatment of early planted seed will be more beneficial.

Barley and wheat seed introduced from the United States and planted in Paraguay in May 1947 was not benefited by seed treatment. Wheat seed produced in Brazil when planted in May or June in peat soil with a high moisture level in the Paraiba Valley was markedly benefited by seed treatment both in terms of germination and subsequent yield of grain. It was noted that the seed of five varieties planted in 1948 carried a high percentage of infection with the scab fungus, *Fusarium* sp.

Hulless peanut seed when treated with seed fungicides gave a markedly higher germination than untreated seed in both Paraguay and Brazil. In one experiment in Brazil the yield from treated seed was significantly higher than from untreated seed.

Seed of rice, beans, and sorghum was not noticeably benefited by seed treatment.

One sample of garden beet seed (Detroit Dark Red) gave about 100 per cent increase in germination when treated with three different dust fungicides.

Seed samples of 10 varieties of peas representing smooth and wrinkled seed types were benefited by seed treatment, although there was evidence that some varieties responded more than others. Seed of the variety Catalana (smooth seeded) when planted in late May or early July in 1947 gave a significantly higher percentage of germination from treated than from untreated seed.

Arasan, Phygon, Granosan, Ceresan, Spergon, and Semesan Jr. were used in the tests. With some exceptions (Semesan Jr. on corn), all were similar in their effects.

RHIZOCTONIA SOLANI IN RELATION TO CEREAL CROWN AND ROOT ROTS

GEORGE W. BRUEHL

(Accepted for publication January 10, 1951)

The action of *Rhizoctonia solani* Kuehn as a vigorous parasite of both shoot and root tissues of cereal crops is indicated by a survey of the literature. A true root rot of wheat and oats caused by *R. solani* has been studied extensively in Australia (4, 6). Australian isolates did not attack culm or leaf tissues. Blair (1) studied some English and Canadian isolates and found those from England to be true root pathogens; the Canadian forms caused a girdling of the wheat ". . . coleoptile or lower stem tissues, usually unaccompanied by adverse effects upon plant growth." The root-parasitizing forms were slower growing than shoot-parasitizing isolates (1) or common *R. solani* from potatoes or broad beans (4). Blair concluded that shoot- and root-attacking forms were at the closest distinct varieties, and that discovery of their sporiferous stages would probably place them in different species. *R. solani* has proved to be a virulent pathogen of shoot tissues in the United States as the cause of brown patch (5) and crown rot and straw-breaking (7) under humid conditions, whereas it has been considered mildly pathogenic or nonpathogenic on cereal roots (8). Ho and Melhus (3) isolated forms in Iowa that attacked the first internode of corn seedlings and eventually caused decortication of the roots, and Ho (2) reported a strain that produced root rot of corn.

Rhizoctonia solani comprised a small percentage of the isolates obtained by the author during routine isolations from root and crown tissues of wheat, oats, and barley from Minnesota, North Dakota, and South Dakota during the spring of 1948. These cultures afforded an opportunity for determining the nature of attack, and whether they were root- or crown-rotting pathogens. Inoculum was prepared by incubating the organism in Erlenmeyer flasks containing 150 gm. of sterile moist black soil at room temperatures (20°–23° C.) for 8 days. The flasks were shaken daily to insure uniform growth through the soils and easy removal of the inoculum. This unit of inoculum was mixed in the top half of sterile soil in unglazed 4-in. pots. Marquis wheat and Gopher oats were seeded at normal depths in the initial tests, using ten isolates. Temperatures in the greenhouse fluctuated about a mean of 20° C., and soil moisture was maintained by estimate at near optimum. Further tests involving several hosts were made under similar conditions. Observations were made on emergence, type of attack, and vigor of survivors.

The parasitism on wheat seedlings included pre-emergence blighting by destruction of the elongating plumule (Fig. 1, A). The rotted plumules of wheat were swollen, soft, watery, and brownish. The severity of attack ranged from total destruction of the young plumule, through noticeable

browning of the coleoptile and lower leaf sheaths, to inconspicuous local lesions. Dry weights of survivors of one series indicated the superficial nature of this basal browning. The roots remained in good condition in the presence of the parasite (Fig. 1, B). In its attack upon oat seedlings pre-emergence killing was similar but less common. The exposed stem tissue (first internode) was frequently the site of a local necrosis (Fig. 1, C). Survivors fared remarkably well. However, such severe injury to the vital first internode, in the field and in the presence of other pathogens, might well serve as an avenue of entrance for them and lead to severe crown rot.

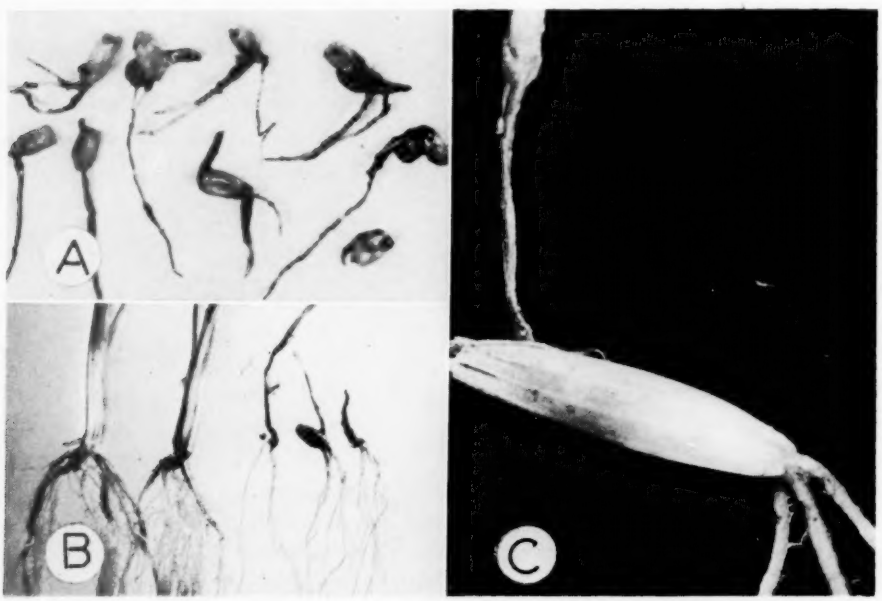

FIG. 1. A. Marquis wheat seedlings attacked by *Rhizoctonia solani:* characteristic destruction of the plumule with seminal roots intact. B. Peliss durum wheat seedlings the same age: escapes (left) and plants blighted by *R. solani* (right) in various stages of emergence. Observe the good condition of the roots of the blighted seedlings, in spite of total lack of photosynthates and obvious extreme conditions of growth. Apparently this fungus failed to damage the roots either by direct invasion or through transfer of toxic substances. C. Exposed plumular tissue of Gopher oats severely damaged; the roots appear unharmed.

The nature of the disease was similar on other members of the Gramineae tested including *Agropyron cristatum, A. trachycaulum, Avena sativa* (four varieties), *Hordeum vulgare* (two varieties), *Triticum durum* (two varieties), *T. aestivum* (eight varieties), *T. timopheevi,* and *Sorghum vulgare*[1]. Among the non-grass hosts tested (*Medicago sativa, Melilotus alba, M.*

[1] Mr. Paul Hoppe, Division of Cereal Crops and Diseases, Wisconsin Agricultural Experiment Station, related in correspondence that an oat isolate of *R. solani* attacked germinating corn, producing seed decay and stunting of surviving seedlings, but when the seed was treated with Arasan the stand was perfect with no appearance of disease until the 3- to 4-leaf stage, when the seedlings began to dry rapidly. Examination showed that the fungus typically had attacked the seedling through the coleoptile, entered the crown, and caused the death of the plant, the roots remaining healthy.

officinalis, Kochia scoparia, and *Linum usitatissimum*), the legume and Kochia seedlings suffered a severe pre-emergence blight, with marked stunting and brownish lesions at the collar region of survivors. Flax seedlings were only mildly parasitized by these isolates.

Several isolates obtained after this study was completed were tested by placing germinated seeds (both roots and plumule emerged) of Mindum durum wheat on plate cultures of the fungus. The roots grew through the agar and mycelium, apparently without injury, while the coleoptiles were matted in hyphae and severely parasitized after about 4 days.

The above observations of the action of cereal isolates of *R. solani* from the hard red spring wheat area indicate that this fungus probably does not play a primary role in root rots of grass hosts in this region, but that it may contribute to the destruction of coleoptiles, lower leaf sheaths, and culm tissues. No positive conclusions can be drawn, however, since true root-rotting forms may be present but undiscovered, and the influence of different environments on the type of attack has not been explored. The genus *Rhizoctonia* varies from true aerial forms entering the hosts through stomata to subterranean parasites of root tissues (9). Extreme variants in type of attack are included in the species *R. solani,* according to the present concept. These isolates of *R. solani* were broad in their host range, but limited sharply as to susceptible organs. These observations substantiate the distinction between shoot- and root-parasitizing forms made by Blair (1).

SOUTH DAKOTA STATE COLLEGE
BROOKINGS, SOUTH DAKOTA

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PHYTOPATHOLOGICAL NOTES

A Correction of the Identification of Helminthosporium Causing Black Ear Rot of Corn. C. D. SHERBAKOFF. In 1937 the writer, together with L. S. Mayer, reported the occurrence of *Helminthosporium turcicum* causing corn black ear rot (Phytopath. 27: 207. 1937). According to later evidence the reported *Helminthosporium* should be *H. carbonum* Ullstrup.—Tennessee Agricultural Experiment Station, Knoxville, Tennessee.

A Method for Large-Scale Inoculation of Sorghum with Colletotrichum. F. J. LEBEAU. In the course of evaluating a large number of varieties, African and Indian introductions, and segregating progenies of sorghum for reaction to anthracnose and red rot, it was found that a reliable rating required the inoculation of many plants in the field and in the greenhouse. Leaf-whorl inoculation with a hypodermic needle and syringe established uniform leaf infection, and the insertion of contaminated toothpicks[1] into stalks established uniform red-rot infection. Because both methods were tedious and required much time and labor, modifications of the hypodermic injections were devised.

A 3-gal. compressed-air sprayer was fitted with a hypodermic needle of gauge 18 or 24. Under a pressure of 20 to 30 lb./sq. in. the spore suspen-

FIG. 1. Diagram of hypodermic needle used for large-scale inoculation of sorghum plants.

sion in the tank streamed out of the needle, which was inserted in the leaf whorl about 6 in. below the top. The pressure was sufficient to eliminate plugging of the needle by plant tissue, and the inoculum oozed between the leaves in the whorl a few seconds after the needle was inserted. More rapid inoculation and less stoppage of the needle was obtained if the stream was permitted to flow when passing from plant to plant, although this procedure wasted over half of the inoculum. Two men could inoculate between 300 and 400 plants in an hour. Three to 4 dozen severely infected sorghum leaves, when kept overnight in a moist chamber at room temperature, provided enough spores for 5 to 10 gal. of a suspension of sufficient density (about 100 spores per low-power microscopic field) to insure inoculation.

The apparatus could not be used for introducing inoculum into stalk tissue because of the excessive plugging of the needle by fibers of the cortical layers. A special needle to eliminate this difficulty was designed (Fig. 1). The open end of a size 12 or 14 needle 1½ in. long was plugged

[1] Unpublished data by M. F. Kernkamp and M. L. Lohman, on file at the U. S. Sugar Plant Field Station, Meridian, Mississippi.

with silver solder and sharpened to a fine point. Four holes (approximately 24-gauge) were bored through the side walls of the needle about ½ in. from the tip at 90° angles and staggered at about ⅛-in. intervals along the length of the needle. This needle was used with the compressed air sprayer for red-rot inoculation in the same manner as described above, except that it was inserted into the stalk tissues and held there momentarily. Only rarely were the holes in the side of the needle plugged. Consistent infection was accomplished by these methods.—U. S. Sugar Plant Field Station, Meridian, Mississippi.

Control of White Root Rot with Chloropicrin in a Border of Ornamental Plants. J. S. COOLEY.[1] A serious outbreak of root rot in 1941, in a planting of ornamental shrubs and herbaceous perennials on "made" land at Silver Spring, Maryland, was due to *Corticium galactinum* (Fries) Burt. The owner of the property stated that the disease had been present for several years and that the affected area was gradually enlarging. The disease first appeared near two maple stumps that were part of the debris used to fill a depression before topsoil had been added; these stumps had probably completely rotted prior to the observations here reported. This same disease has been noted by Cooley and Davidson in apple orchards,[2] and in ornamental shrubbery plantings. In these cases the initial infection has usually been associated with infected stumps.

In September 1941, when a careful census was made of the affected plants growing in the shrubbery border, red cedar (*Juniperus virginiana*) was the only woody plant in the infested zone that was not affected by white root rot. The plants affected were: *Baptisia australis* (blue wild-indigo); *Exochorda racemosa* (common pearlbush); *Iris*, Pogoniris group (tall bearded iris); *Jasminum nudiflorum* (winter jasmine); *Paeonia officinalis* (common peony); *Prunus glandulosa* (flowering almond); *Prunus triloba* (double-flowering plum); *Spiraea thunbergii* (Thunberg spirea); and *Viburnum carlesi* (fragrant viburnum).

None of these plants have been previously reported as affected by this disease. In previous work by the author, as well as in the present case, there is evidence that the disease is not restricted to a particular group of plants, but is likely to attack a wide range of plants if they happen to be growing in infested soil.

All plants were removed from the infested zone and for 2 or 3 ft. beyond where the plants showed any evidence of diseased roots. This made a plot of 12 ft. × 20 ft., or 240 sq. ft. On September 3, 1941, chloropicrin was injected into the soil at staggered 1-ft. intervals (2 ml. per injection) throughout the infested area. The soil was thoroughly wetted

[1] Senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Maryland.

[2] Cooley, J. S., and Ross Davidson. White root rot of apple trees caused by *Corticium galactinum*. Phytopath. 30: 139–148. 1940.

after the application of chloropicrin and then covered with water-impervious paper[3] to retard the escape of the gas from the soil. The margins of the paper were buried to a depth of 16 in. to further insure retention of the gas.

In the spring of 1942 the following woody plants were planted in the plot: *Acer palmatum* (Japanese-maple); *Aronia arbutifolia* (red chokeberry); *Babtisia australis* (blue wild-indigo); *Buddleia davidi* (orange-eye butterflybush); *Euonymus americanus* (brook Euonymus); *Euonymus fortunei* var. *vegetus* (bigleaf wintercreeper); *Jasminum nudiflorum* (winter jasmine); *Juniperus horizontalis* (creeping juniper); *Malus sieboldi* (Toringo crabapple); *Polygonum auberti* (China fleecevine); *Prunus glandulosa* (flowering almond); *Prunus persica* var. *duplex* (double-flowering peach); *Rosa moschata* (musk rose); *Spiraea prunifolia* (bridalwreath spirea); *Taxus media* var. *hicksii* (Hicks yew).

This planting included three species that were affected with white root rot in the original planting and two other species (*Malus sieboldi* and *Prunus persica*) known to be susceptible to the disease.

In the spring of 1947 a second planting was made with the following plants: *Deutzia gracilis* (slender deutzia); *Exochorda racemosa* (common pearlbush); *Iris,* Pogoniris group (tall bearded iris); *Kerria japonica* (Japanese kerria); *Lythrum salicaria roseum* (rose-colored lythrum); *Paeonia officinalis* (common peony); *Philadelphus sp.* (mockorange); *Physocarpus opulifolius* (common ninebark); *Prunus triloba* (double-flowering plum); *Sophora japonica* (Chinese scholartree); *Spiraea nipponica* (Nippon spirea); *Syringa chinensis* (Chinese lilac); *Viburnum opulus* (European cranberrybush).

In the two replantings seven of the nine species originally affected were set out in the treated area, and in addition, two species known to be susceptible and 17 species of unknown susceptibility. At intervals from 1942 to December 1949 inspections were made in and adjacent to the treated area, but no evidence of white root-rot infection was observed.

The observations reported in this paper indicate that white root rot may attack a wide range of ornamental shrubs and herbaceous perennials. Most of the species or varieties of plants that were affected by the disease before soil treatment with chloropicrin were planted back after the treatment. Also, other woody plants, some of known and others of unknown susceptibility to the disease, were planted in the treated area. The absence of the disease on any of these plants after 2 to 7 years of growth indicates that a localized area infested with white root rot can be successfully treated with chloropicrin for eradication of that disease.—Plant Industry Station, Beltsville, Maryland.

[3] This paper is the kind used by nurserymen for packing plants and consists of a layer of asphalt between two layers of brown manila paper.

REPORT OF THE 42ND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its forty-second annual meeting at the Hotel Peabody, Memphis, Tennessee, on December 1, 2, and 3, 1950. The Council met on November 30 and December 1. Business meetings were held on December 1 and 3. The Southern Division met with the Society. During the afternoon of December 3, a joint session was held with the Potato Association of America. Four hundred and sixteen members and guests registered. One hundred and eighty-nine papers were scheduled for presentation in twenty sessions and symposia. Symposia and conferences were conducted under the following titles: Stone Fruit Virus Diseases, Extension and Research Relationships from Different Viewpoints, The Teaching of Plant Pathology, Fungicide Colloquium, Should the Plant Disease Survey Revive Crop Loss Estimates, and Application of Latest Research Findings to the 1951 Extension Program.

The annual dinner held at the Peabody Hotel was attended by approximately 325 members and guests. Entertainment was provided by the Peabody Chorus. A brief program in honor of Dr. B. M. Duggar, a pioneer in plant pathology in the United States, was held in conjunction with the dinner. Friends of Dr. Duggar presented him with a bound volume of letters and a gift.

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Report of the Secretary. On November 22, 1950, the total membership was 1,503, consisting of 141 life members, 1232 regular members, and 130 applicants. Reports were received of the death during the year of two members, Raymond A. Dobbins and Luther P. Byars (life).

Report of the Treasurer. Statement of accounts for the year October 1, 1949, to September 30, 1950.

Receipts:		
Balance from previous year	$ 6,770.57	
Annual dues; subscriptions; miscellaneous	8,667.65	$15,438.22
Expenditures:		
Transferred to Phytopathology	$ 5,649.00	
Dues to AIBS, 1949–1950	900.00	
Office of the Secretary	348.15	

Office of the Treasurer	857.68	
Exchange on Canadian check	1.23	
Committee expenses	49.94	
Miscellaneous	27.00	
	7,833.00	
Balance on hand, September 30, 1950	7,605.22	$15,438.22

A. E. DIMOND, *Treasurer*

Report of the Business Manager for Phytopathology. Statement of accounts for the year October 1, 1949, to September 30, 1950.

Receipts:

Balance from previous year	$ 7,788.55	
Subscriptions	10,507.41	
Member subscriptions and sales transferred from APS	5,649.00	
Advertisements	3,077.07	
Sales, back volumes	2,817.75	
Reprints, excess illustrations	3,313.97	
Sustaining Associates, grants and donations	2,050.50	
Miscellaneous	19.79	
Interest on sinking fund	31.50	
Interest on investments	30.00	$35,285.54

Expenditures:

Manufacture and distribution of Phytopathology	$17,851.49	
Reprints	2,156.31	
Office of the Editor-in-Chief	1,768.73	
Office of the Business Manager	565.21	
Office of Advertising Manager and Agency Commission	456.22	
News Letter	276.56	
Miscellaneous (refunds, etc.)	280.82	
	23,355.34	
Balance on hand, September 30, 1950	11,930.20	$35,285.54

Sinking Fund: The principal of the sinking fund remained the same as for the previous year.

First mortgage note, at 3.8 per cent interest, deposited with McLachlen Banking Corp. for collection	$ 500.00	
U. S. Savings Bond, Series G, No. M1905602G, 2½ per cent interest	1,000.00	
Northwestern Federal Savings & Loan Ass'n (certificates)	2,000.00	
War savings bonds, Series F (maturity value)	1,150.00	
Jefferson Federal Savings & Loan Ass'n (accrued dividends $140.64)	640.64	
District Bldg. Loan Ass'n (accrued dividends $417.49)	1,917.49	
National Permanent Bldg. Ass'n (accrued dividends $698.54)	2,698.54	
Perpetual Bldg. Ass'n (accrued dividends $281.35)	1,281.35	
Prudential Bldg. Ass'n (accrued dividends $60.26)	266.60	
Arlington and Fairfax Bldg. & Loan (accrued dividends $177.86)	1,177.86	
	$12,632.48	
Less interest due Phytopathology	1,776.14	$10,856.34

Lyman Memorial Fund: This fund is obtained from voluntary contributions and now totals $4,167.32, including interest. This amount is invested with the Brookland Bldg. and Loan Ass'n at 2½ per cent interest.

Balance on hand September 30, 1949	$4,065.08		
Dividends December 31, 1949, and June 30, 1950	102.24	$4,167.32	
Less interest due Phytopathology		783.89	$3,383.43

Phytopathology Contingency Fund:

Northwestern Federal Savings & Loan Ass'n:		
Balance on hand October 1, 1949	$5,501.94	
Interest	196.74	$5,698.68

A. E. DIMOND, *Business Manager*

Report of the Auditing Committee for the Fiscal Year, 1949–1950: We have examined the books of the Treasurer of The American Phytopathological Society and of the Business Manager of Phytopathology for the period from September 30, 1949, to September 30, 1950. We found all receipts and expenditures and all funds of this Society and of Phytopathology accurately recorded.

Signed: ERNEST M. STODDARD
FLOYD L. WINTER

Report of the Advertising Manager, 1950. Eighty-four and one-half pages of Phytopathology were used by 103 advertisers, bringing a gross income of $3,600.80. The income to the Society after deduction of agency commissions and cash discounts to advertisers was $3,121.87. Expenses of the advertising manager were: telegraph and postage, $28.16; clerical, $72.00; commissions, $50.00 (5 per cent of $1,000) and $212.19 (10 per cent of $2,121.87); total, $362.35. These reduced the returns to the Society to $2,759.52.

Thirteen pages were used for gratis advertisements: 3.5 for Biological Abstracts, 3.5 for the Placement Committee of the Society, and 6 for Phytopathological Classics.

L. J. ALEXANDER, *Advertising Manager*

Report of the Editor-in-Chief. The fortieth volume of Phytopathology contains 1159 pages wherein 398 authors published 108 articles, 27 Phytopathological Notes, 3 biographies, 25 book reviews, 136 abstracts presented at the annual meeting of the Society and its Northeastern Division, 21 abstracts for the meeting of the Potomac Division, 28 abstracts for the Southern Division, and 37 abstracts for the Pacific Division. The annual report, one announcement, one notice regarding common names for fungicides, and one notice about the National Scientific Register were published for the Society. Between November 30, 1949, and November 30, 1950, eighteen manuscripts were rejected or were withdrawn for publication elsewhere.

The number of manuscripts submitted between May and November, 1950, is exceptionally high. On November 15 the material on hand was: 33 manuscripts in press or in galley proof: 44 manuscripts (514 typescript pages) approved by reviewers and in editorial process, 17 manuscripts in need of authors' revisions, and 19 manuscripts submitted to reviewers. Enlarging some of the early issues in 1951 is expected to reduce the large backlog of papers awaiting publication, but the ever-increasing costs of publication must be countered by greater condensation of manuscripts and more careful selection and organization of material by authors.

The change to double-column format contemplated for 1951 has been postponed to 1952. Specifications for illustrations and tabulated material will be published in Phytopath News during the first half of 1951.

A Ten-Year Cumulative Index for 1941–1950, for volumes 31 through 40, will be published early in 1951.

The Journal is pleased to acknowledge the continuing support of the Sustaining Associates of the Society, the financial contribution from the Rockefeller Institute for Medical Research, the preparation of the current index by Associate Editor Thor Kommedahl, and the valuable editorial assistance of Mrs. Ralph H. Brown.

HELEN HART, *Editor-in-Chief*

Report of the Editor of Phytopath News. It is anticipated that five numbers of NEWS will be published and distributed to members of the Society in 1951. The last two numbers were delayed because the editor moved from State College, Pennsylvania, to Beltsville, Maryland. Costs of publication have remained reasonably stable and, barring unanticipated increases in expenses, the $300 previously budgeted should suffice for publication and distribution in 1951.

K. W. KREITLOW, *Editor*

Report of Representative to National Research Council, Division of Biology and Agriculture. The annual meeting of the Division was held in Washington on May 9, 1950, and a full report was mimeographed and distributed to members of the Council. The activities of the Division of particular interest to APS may be summarized briefly as follows:

Arrangements have been made for publication by McGraw-Hill of a three-volume revision of the monograph on Radiation in Biology. The first volume is expected in 1951.

Committees are maintained to screen applications for Fulbright awards.

In the reorganized Agricultural Board, phytopathology is represented by E. C. Stakman.

AIBS continues to receive the support and sponsorship of the Division. The activity of AIBS is given in a separate report.

J. C. WALKER

Report of Representative on the Governing Board of American Institute of Biological Sciences. The annual meeting of the governing board was held on May 8, 1950, and a full report was mimeographed and distributed to the Council. The major accomplishments of AIBS during the year may be summarized briefly as follows:

A full-time secretary, C. J. Hylander, has been engaged and clerical assistance has been set up, with offices in space provided by the Carnegie Institution in Washington.

In anticipation of the organization of National Science Foundation a comprehensive survey of biological research and future needs was made and placed in the hands of the Budget Bureau. The report states that about $6,000,000 per annum could be spent advantageously by the Foundation on biological research in universities and agricultural colleges.

A self-sustaining annual meeting of 14 biological societies at Columbus, Ohio, was sponsored in September. A similar meeting is being arranged for September, 1951, at Minneapolis.

A plan was sponsored in behalf of Biological Abstracts whereby $20,000 was provided by the Office of Naval Research to expedite prompt publication of the 1949 and 1950 indices.

Through the activity of members of AIBS on an advisory committee to General Hershey regarding scientific manpower, a comprehensive national roster of biologists is being assembled to include graduate students.

The Committee on Publication Problems conducted a half-day symposium on Sept. 13, 1950, at the Columbus meeting.

J. C. WALKER

Report of Representative on Board of Editors, American Journal of Botany. No manuscripts were presented for examination during the year.

CHARLES CHUPP

Report of the Committee on Donations and Legacies. This Committee interpreted the action of the Council at the New York meetings in December, 1949, as tacit approval of its proposal to seek a wider base of contributions to the Society's endowment fund by a general appeal to the membership. Accordingly, an insert soliciting such contributions was mailed to the members along with the notice of dues for 1951.

Since a relatively small proportion of the members pay their dues prior to the date of the annual meeting, it is too early to appraise the success of this solicitation. The Society Treasurer has informed us, however, that at the close of the fiscal year, September 30, he had deposited $100 from this source to the account of PHYTOPATHOLOGY, and he stated that, as of October 13, $41 additional had been received. Doubtless further contributions have come in since then. The individual donations ranged from $3 to $50. It appears that the solicitation has at least partly accomplished its purpose.

Some of the Society members have protested that this course puts the Society in the position of a mendicant, and they would prefer an outright increase in the dues in order to give the Society the financial support it needs. With the question of raising the dues this Committee has no conern, but it believes that the matter of supporting certain activities of the Society, such as furnishing PHYTOPATHOLOGY gratis to individuals and institutions in dollar-short countries or in difficult circumstances otherwise, is best managed by voluntary contributions. Likewise, certain publication problems of the journal, such as admitting papers by nonmembers and allowing extra pages and illustrations for meritorious articles, should be resolved by asking those who can most readily (subject to their individual judgment) pay the cost rather than impose it upon all members, some of whom are disinclined or financially unable to bear it. The real test of the validity of this policy would seem to be whether the desired supplementary income can be obtained from voluntary contributions. Perhaps the policy should have at least one more year's test before a decision for or against it is made.

It should be noted that the Treasurer has credited the contributions from this source to the account of PHYTHOPATHOLOGY instead of to the fund from donations and legacies because the latter is an encumbered fund. The Council should, perhaps, fix a policy for allotting funds received from this source, since some of the contributors may want only the income from these funds used for the purposes mentioned.

Dr. Pirone, at the New York Botanical Garden, has assumed the task of keeping a record of persons and institutions in foreign countries that have requested preferential treatment in maintaining their subscriptions to PHYTOPATHOLOGY, and also of all subsidies made by the Society or by individual members in their behalf.

FREEMAN WEISS, *Chairman*

Report of the Extension Committee. There were 18 registered in attendance at the Extension Section in the McAlpin Hotel, most of whom were extension pathologists.

The theme of the conference was: "What's New in Disease Control?" Dr. R. J. Haskell gave the keynote talk on the relation of the extension specialists in plant pathology to their subject-matter department. This was based on replies to a questionnaire that had covered most of the States. This was not only informative, but pointed out the trends as well as the manner in which extension specialists can advance our special field. The group requested that this material be published for further study. This has been done and the material has been distributed. The remainder of the program was taken up in discussing new methods of teaching and furthering 4-H Club work, after which recommendations and discussion of results from various States were considered. The subject groups considered were: 1) Orchard fruits; 2) small fruits; 3) ornamentals, turf; 4) vegetables, potatoes; 5) tobacco, cotton, peanuts, sweet potatoes; 6) cereals, forage crops. It was pointed out by those present that these discussions were very helpful in appraising different recommendations for local use by extension people. Some members of the group brought problems with which they had been confronted during the year.

An informal meeting during the dinner hour was held between the adjournment of the group and the time scheduled for the evening papers. The following day a call meeting was held for determining what trend the program should take for the next year. The decision reached there has been ratified by the remainder of the Extension Committee by correspondence. In formulating plans for the coming year it was decided to continue the present plan of summarizing recommendations on the basis of subject matter and to expand consideration of extension-research relationships.

J. O. ANDES, *Chairman*

Report of the Committee on Finances and Investments. The Committee is studying the financial structure of the Society and its investments with a view to obtaining greater returns from invested funds but without reduction in security. The Committee wishes to continue and extend its investigation before making a formal report.

W. H. TISDALE, *Acting Chairman*

Report of the Business Manager, Phytopathological Classics. During the period from October 1, 1949, to September 30, 1950, 219 copies of Classics were sold, having a total value of $192.00. On the latter date there were on hand 84 copies of Classic No. 2; 165 of No. 3; 232 of No. 4; 450 of No. 5; 530 of No. 6; 494 of No. 7; and 669 of No. 8.

Receipts:			
Cash balance October 1, 1949		$627.13	
Received during fiscal year 1949–1950:			
For Classics sent out	$191.50		
For accounts due from previous years	13.50	$205.00	$832.13
Disbursements:			
Postage	$ 17.76		
Typing copy of translation for proposed Classic No. 9	44.00	61.76	
Cash balance September 30, 1950		770.37	$832.13

Due on accounts September 30, 1950: $23.50.

L. J. TYLER, *Business Manager*

Report of the Editor of Phytopathological Classics. The next publication in the series of PHYTOPATHOLOGICAL CLASSICS will be a translation of the "Alimurgia" by Targlioni-Tozzetti. This eighteenth-century work, according to Count Re, contains the first elaborated suggestion that plant diseases are caused by specific organisms. It is a large work, carefully thought out and neatly executed within the limits of its epoch, and through its annotated discussion can be glimpsed the phytopathological thought of the times, not only that of its author but of his contemporaries as well. The writings of Tozzetti, significant as they are in the history of pathology, remain relatively unknown in the United States, and it is the hope that this gap in an understanding of our antecedents can be closed by a reprinting of the "Alimurgia" in English. The translation from the Italian edition of Professor Gabrielle Goidanich is being made by Dr. L. R. Tehon, and Classic No. 9 should be off press during 1951.

In view of the editor's temporary domicile in Argentina, it may prove expedient to have the printing done in that country because of the typographical editing necessary to insure a faithful reproduction of the original. In the event that quality of work and cost including freight are comparable to those in the United States, it is the wish of the editor to proceed according to the dictates of circumstances, without, however, overlooking any objections from the membership. If such do occur either to the officials or to the rank-and-file, their transmission would be appreciated to the Estacion Experimental Citricola, Concordia, Entre Rios, Argentina.

L. C. KNORR, *Editor*

Report of the Placement Committee. During 1950, fifty-two plant pathologists had applications in the active files of this Committee. In an attempt to fill 30 positions, 154 requested applications were sent out to prospective employers. Incomplete records show that at least three plant pathologists obtained positions through the efforts of the Committee.

Dr. R. S. Kirby, Chairman of the Placement Committee, left on sabbatical leave July 15, 1950; since then the Acting Chairman has continued the service of the Committee.

E. E. HONEY, *Acting Chairman*

Report of the Committee on Public Relations. The Committee carried on the usual line of effort in 1950 arranging publicity for the New York meetings held in conjunction with AAAS, encouraging publication of articles for laymen, and setting the stage for the Memphis meetings. The outstanding achievement of the 1949 meeting was the Committee's choice of Dr. Armin C. Braun's paper on mechanics of growth in crown-gall tissue, and its promotion through the proper channels of the AAAS, for receiving the Association's $1,000 prize for an outstanding paper. Through the Committee's auspices it was also possible to arrange time for our members on one television and three radio programs and to transmit at least six noteworthy news items to the metropolitan newspapers.

During the intervening months, the Committee has aided authors and editors or written articles designed for the vernacular press as follows. Data were assembled and conferences arranged with Editor Steven Spencer of the Saturday Evening Post for an article on tree diseases which has not been released. Twelve articles on the more important diseases of fruits and vegetables are being compiled for publication in The Canning Trade. Articles were also published on plant diseases in the American Fertilizer (1), Agricultural Chemicals (each issue), California Fruit and Grape Growers (5), California Citrograph (5), Citrus Leaves (1), California Farmer (1), California Agriculture (4), newspaper articles (15) in Pennsylvania, What's New in Crops and Soils (republished in Newsweek), Plant Disease Reporter (3), New International Year Book for 1950, Papers of the National Chemurgic Council (1), American Fertilizer (1), New York Times (2), Baltimore Evening Sun (1), Pennsylvania Farmer (1), Southern Florist and Nurseryman (1), Southern Agriculturist (1), Better Crops with Plant Food (1), and Country Gentleman (1).

Arrangements were made for the 1950 Annual Meeting through the Memphis Chamber of Commerce. Radio stations and the daily newspapers were apprised of the meetings and some of the background on special papers.

GEORGE L. MCNEW, *Chairman*

Report of Committee on International Cooperation. The Committee continued its attempts to stimulate the flow of literature from the United States to other countries, including particularly Japan and the Philippines.

The question of international cooperation was discussed at the meeting of the phytopathological section of the Seventh International Botanical Congress at Stockholm in July. There was unanimous agreement that it would be desirable to make rather definite arrangements for cooperation in a number of specific fields. To implement these suggestions it was voted to continue the officers of the Stockholm congress as an international Committee to promote the objectives of international phytopathology. Because of the fact that another committee has nominally been in existence since the Amsterdam congress in 1935, the status of that committee must be determined. Attempts now are being made to obtain this clarification.

In the meantime, certain proposals regarding international phases of plant pathology have been made by one of the officers of the Union of Biological Sciences.

A definite step toward closer cooperation in the Americas was taken at a conference held in Mexico City, September 25 to October 6, 1950. This was known as the Primera Asamblea Interamericano de Fitoparasitologia and was held under the joint auspices of the Ministry of Agriculture of Mexico, the Rockefeller Foundation, and the governments of the countries represented. The meeting was attended by plant pathologists, mycologists, and economic entomologists. The group authorized the appointment of a committee of pathologists and entomologists to be known as the Interamerican Committee on Plant Protection. The general objectives are to promote the rapid and systematic exchange of information regarding pests and pathogens, and regarding fungicides and insecticides; to make uniform tests of fungicides and insecticides in different countries and under different conditions when and where feasible; to exchange information regarding special methods or materials used in investigations; and to arrange for uniform trials of plant varieties in order to determine their reactions to pests and diseases. It was proposed, furthermore, that the Committee study and promote all means of cooperation. Among other provisions was one requesting the Interamerican Committee to seek some

sort of affiliation with the APS Committee on International Cooperation in the hope that one or more members of the Interamerican Committee might be members of the APS Committee and thus provide for as much coordination as possible. This proposal has not yet been acted upon by the APS Committee nor by the Council of the Society, but it is very gratifying that representatives from the various American countries so spontaneously and enthusiastically agreed to do everything within their power to bring about more effective cooperation among the various countries.

There have been consultations with officials of the Food and Agriculture Organization of the UN with a view to helping the FAO in their program of attempting to develop international cooperation in the control of pests and pathogens.

E. C. STAKMAN, *Chairman*

Report of the Committee on Plant Disease Prevention, and Subcommittee on Regulatory Work and Foreign Plant Diseases. The activities of this Committee during the past year have dealt for the most part with problems of national defense. The Subcommittee on Utilization of Plant Pathologists and Facilities in National Emergencies has been especially active. The report of this Subcommittee is attached hereto.

The Subcommittee on Regulatory Work and Foreign Plant Diseases has made two recommendations to the Council since the last annual meeting; one to the effect that a motion picture record of the methods used in eradicating potato wart in Pennsylvania should be made, and another relating to the desirability of including cereal seeds among the plant products excluded from entry into the country. The chairman of this Committee is out of the country and no formal report is being made.

The report of the Subcommittee on Seed and Plant Material Certification is given separately.

The responsibilities of the Committee relating to "the need for effective protection of major crops and trees against plant diseases with particular reference to national defense" was handled in 1949 by a special Subcommittee as directed by the Society at the 1948 annual meeting.

During the past year, because of security regulations, the chairman of this Committee alone has represented the Society in official conferences on the subject. Other members of the Committee and of the Society, however, have usually been present at such conferences in other capacities. All that can be reported at this time is that responsible authorities at high levels have been convinced that there is great need for more effective protection of crops and that an adequate survey and plant disease reporting service should be an important part of our civil defense program. The first plans for civil defense were drawn up under a directive from the Secretary of National Defense. The recommendations submitted in response to this directive included our recommendations in all essential details. Direct action on these recommendations was somewhat delayed pending a high level decision in the federal department which would be responsible for civil defense activities. Essentially the same recommendations relating to plant disease prevention were later submitted to the National Security Resources Board, now responsible for Civil Defense, and have been favorably received.

The Chairman of your Committee has every reason to believe that the Civil Defense program now being worked out by the National Security Resources Board will include a comprehensive program of plant disease prevention, and that when it is put into effect it will not be crippled by secrecy restrictions.

J. G. LEACH, *Chairman*

Report of the Subcommittee on Utilization of Plant Pathologists and Facilities in National Emergencies. The subcommittee by correspondence and conferences has been active on the following considerations:

Joint conference between military personnel and plant pathologists. Dr. H. L. Buckardt (Personnel Policy Board, Office of the Secretary of Defense) invited Dr. H. T. Cook and Mr. H. P. Barss to attend the August 8 meeting of this Personnel Policy Board. Three reserve officer plant pathologists (H. T. Cook, Navy; H. Rex Thomas, Air Force; and H. H. Thornberry, Army) are being considered for two weeks of active-duty assignments to work up job descriptions and qualifications for plant pathologists in the three military departments.

Eight military job titles and descriptions (continued from 1949).

Classified directory of Civilian Specializations, Military Specializations, Personnel, Facilities, and "Know-Hows" in plant pathology.

A directory of sources of grants-in-aid to phytopathological research pertinent for national defense is being compiled by Dr. A. L. Anderson.

A plan for the establishment and administration of a National Phytopathological Research Program (New Horizons in Phytopathological Research) pertinent to national defense.

Present military mobilization as follows: (a) Inquiries from individuals. (b) Request from the National Security Resources Board to APS for recommendations relative to the mobilization of scientists in national defense. A special report has been sent to Dr. J. G. Leach, chairman of the Committee on Plant Disease Prevention.

The Committee recommends that The American Phytopathological Society: 1. Continue this Committee for 1951. 2. Continue to assist this Committee in its efforts to bring about joint conferences between some military personnel and plant pathologists for the purpose of providing for effective utilization of plant pathologists in national emergency. 3. Authorize this Committee to contact the personnel and agencies necessary for preparing a directory of the sources of grants-in-aid for phytopathological research pertinent to national defense. 4. Encourage AIBS to appoint a committee on the utilization of biological scientists in national emergency.

H. H. THORNBERRY, *Chairman*

Report of Subcommittee on Plant Disease Prevention, Committee on Seed and Plant Material Certification. The procedure of seed and plant material certification affords a unique opportunity to utilize certain well established, effective, and desirable disease control measures. Most seed of new field crop varieties developed in this country, and new introductions from other countries, are initially distributed to growers as certified seed. Seed of new varieties developed by testing and selection in a "disease garden," and resistant to prevalent races of seed-borne pathogens, may be infected with virulent races not previously present in a crop area. New races and pathogens likewise can be introduced from other sections of this country and from other countries.

This Committee wishes to go on record in favor of recommending to the International Crop Improvement Association that seed treatment by an effective fungicide be made one of the requisites for the production of foundation and registered seed stocks of field crops where satisfactory treatment methods are available and justified. It is suggested that a joint committee of APS and the International Crop Improvement Association be appointed to develop specific recommendations relative to seed-treatment requirements for the production of foundation, registered, and certified seed. This same committee might want to make similar recommendations to the various State crop improvement associations.

It is further recommended, in view of the critical need for more information concerning plant pathogens in foreign countries, that additional emphasis be placed on Item 20 recommended by the Council and approved by the Society as stated in the Report of the 39th Annual Meeting (Phytopath. 38: 325. 1948).

H. C. MURPHY, *Chairman*

Report of the Committee on Resolutions. The American Phytopathological Society expresses its sincere thanks:

To J. O. Andes, Chairman, W. W. Hare, J. M. Epps, Coyt Wilson, and their assistants for their excellent efforts in the preparation of local arrangements for the meeting;

To the management and staff of the Hotel Peabody for their courteous and considerate efforts on behalf of the Society and its members;

To the Memphis Convention Bureau for efficient assistance at the Registration Desk;

To the Peabody Jubilee Singers for entertainment at the annual dinner; and

To President Tucker, Vice-President Horsfall, Secretary May, Treasurer Dimond, and Editor-in-Chief Hart for their efforts on behalf of the Society during the year.

S. J. P. CHILTON, *Chairman*
H. E. WHEELER
C. H. KINGSOLVER

Elections and Appointments. A committee of the Council opened and counted the ballots for President, Vice-President, and Councilor-at-large. J. G. Horsfall, G. L. McNew, and G. C. Kent were elected, respectively, to these offices. S. J. P. Chilton was selected by the Council to fill the unexpired term of Councilor-at-Large G. L. McNew, who was elected Vice-President.

The Council recommended and the Society approved the appointment of S. E. A. McCallan as Editor for 1951. 1952, and 1953, and S. P. Doolittle, Randall R. Kincaid, H. R. Rosen, and George A. Zentmyer as Associate Editors for 1951, 1952, and 1953.

The Council and the Society express their appreciation of the editorial work voluntarily contributed to the Society by the following whose terms expired December 31, 1950; Editor James H. Jensen, Associate Editors Donald Cation, J. G. Harrar, K. W. Kreitlow, and J. D. Wilson.

L. J. Alexander was reappointed Advertising Manager of PHYTOPATHOLOGY for 1951; L. C. Knorr and L. J. Tyler were reappointed as Editor and Business Manager, respectively, of PHYTOPATHOLOGICAL CLASSICS for 1951; J. C. Walker continues to represent the Society as Director on the Governing Board, AIBS; Curtis May was

appointed representative to the Division of Biology and Agriculture, National Research Council, for 1951; C. M. Tucker was appointed representative on the AAAS Council for 1951–1952; A. J. Riker was appointed representative on the Board of Editors, American Journal of Botany, for 1951; H. H. McKinney was continued as representative to the American Type Culture Collection; and H. H. Thornberry was continued as representative to the Associates Food and Container Institute; G. L. McNew was appointed representative on the Food Protection Committee of the Food and Nutrition Board, National Research Council; C. L. Lefebvre was appointed representative to the National Conference of Non-governmental Organizations on Food and Agriculture Organization.

Interim and Ad Hoc Appointments. The President made the following interim and ad hoc appointments during the year:

B. L. Richards to represent the Society at the inauguration of L. L. Madsen as President of Utah State Agricultural College;

Karl Silberschmidt to represent the Society at the International Microbiological Congress, Rio de Janeiro;

L. M. Black as chairman of the Plant Virus Section of the subcommittee on Virus Nomenclature, International Botanical Congress, Stockholm, Sweden;

C. L. Lefebvre and H. P. Barss as Society representatives to a meeting of Non-governmental Organizations interested in the Food and Agriculture Organizations, United Nations; and

L. M. Hutchins as Society representative to the May 1950 meeting of the Scientific Conference on the Conservation and Utilization of Resources of the United Nations.

Reports of Officers, Representatives, Standing Committees, and Interim appointments as published in this report, and reports of Special and Temporary Committees (not published) were approved by the Council and accepted by the Society.

The following actions were recommended by the Council and approved by the Society:

1. Committee assignments, new members of the Editorial Staff of Phytopathology, and Society Representatives as presented.
2. All applicants for membership were accepted.
3. Authorized the Subcommittee on Seed and Plant Material Certification to investigate the possibility of organizing a joint committee with the International Crop Improvement Association to develop a program of seed treatment requirements for registered, certified, and foundation stocks. The Subcommittee is authorized to represent the Society on the proposed joint committee.
4. Approved the selection of S. E. A. McCallan as Secretary for 1951–1953.
5. Approved the purchase of an electric typewriter by the Business Manager of PHYTOPATHOLOGY.
6. Approved changing the annual dues of members from $7 to $10 per year beginning with the year 1952.
7. Approved an increase in the subscription rate to PHYTOPATHOLOGY to $10 per year beginning January, 1952.
8. Approved an assessment of $3 per member for 1951 to meet increased publication costs of PHYTOPATHOLOGY.
9. Approved a resolution nominating Dr. J. G. Horsfall chairman of a special committee to correspond with life members regarding voluntary contributions to the Society.
10. Approved monthly publication of not more than one page of News Letter in the advertising section of PHYTOPATHOLOGY and the discontinuance of separate printing and mailing of the News Letter.
11. Approved publication of a decennial index of PHYTOPATHOLOGY.
12. Instructed the Council to appoint necessary committees to investigate the feasibility of the establishment by the Society of a popular plant disease journal.
13. Approved a resolution instructing the Council to consider a joint meeting with the AAAS in 1952.
14. Approved in principle for further action of the Council the prior action of the Potomac Division regarding the establishment of a Federal research journal.
15. Approved the following resolutions in respect to the publication of the results of scientific investigations:

RESOLUTION supporting the establishment of a Federal journal for publication of research in biology and agriculture. The publication of scientific information, especially in biology, is in a critical position because of the obvious paucity of scientific journals available to publish the valuable data resulting from research. Hence, the printing of new knowledge lags far behind its acquisition. This situation reveals two results, neither of which is good: 1) the stimulating effect of new knowledge on active research

is lost, and 2) costly effort is often totally wasted because two or more researchers may be investigating the same field in ignorance that others are doing much the same thing.

Government has left this significant aspect of science largely to the professional societies, and the researchers must pay from their own purse the costs of publication. This undue pressure on the professional societies has become so great that their journals can no longer publish the results of research promptly. The publication of the results of research is clearly part of the research itself and scientists should no more pay for publishing their research data than they should pay for the apparatus that they use in their experiments.

The American Phytopathological Society hereby earnestly requests the Interagency Committee of the Federal Government to (a) re-examine policies on publication, (b) establish and support a suitable journal or journals in the fields of biology and agriculture, and (c) arrange to defray the costs of publishing the results of research in technical journals.

JAMES G. HORSFALL
C. L. LEFEBVRE

RESOLUTION stating the position of the Society with respect to the responsibility of institutions for the publication of results of research. The rapid increase in scientific research in recent years has created a serious problem in publication that promises to become worse unless some radical changes are made in traditional publication practices. In early days, when research was largely a by-product of teaching, there was very little money available for research and little or no provision for publishing the results of research. In order to meet the growing demand for publication facilities, the various scientific societies established journals supported by membership dues. As more and more research was done, new societies were organized and new journals were established. Additional outlets were provided in the bulletins of the State agricultural experiment stations and of the United States Department of Agriculture. However, because of the limited and ineffective distribution of bulletins, more and more of the results of research have been published in the journals. With the intensified interest and support of research since the second world war, the burden of publication has become too great for most of the scientific societies so that many valuable articles cannot be published promptly or at all.

It is well known that in most scientific societies 80 per cent or more of the membership dues is required for the publication of the journal. Thus the research workers are paying from personal funds a large part of the cost of publishing the results of their research. There is no reason why they should be required to do this any more than they should be required to pay for the chemicals and glassware used in doing the research. The worker who writes the article for publication is not the one who profits from its publication. The paper is valuable only to the reader who uses the facts presented and to the general public that benefits from their use.

Since much of the financial support now being made available for research from various sources makes no provision for paying the cost of publications in journals, the burden on the scientific societies is steadily increasing. In view of this critical situation, The American Phytopathological Society at its annual meeting at Memphis, Tennessee, on December 1, 2, and 3, 1950, adopted the following recommendations:

1. That all agencies responsible for administering research funds take steps to see that adequate provisions are made for paying the costs of publication of the results of the research.

2. That since such a large proportion of the results of research is published in scientific journals financed entirely by membership dues, some means be devised and approved by which some of the cost of publication can be borne by the agency sponsoring the research.

3. That since this problem is acute in most of the biological societies it be given special attention by the American Institute of Biological Sciences.

4. That should the Advisory Board of the National Science Foundation recognize scientific journals as an avenue of publication of research financed by the Foundation, some provision be made for bearing the cost of such publication by the Foundation.

J. G. LEACH

The Secretary is instructed to send copies of the above resolutions to appropriate private and public research institutions and organizations.

16. Accepted the changes in Articles 4 and 5 of the Constitution as published in PHYTOPATHOLOGY, Vol. 40, No. 9, September, 1950; said changes providing for a President-Elect as an officer of the Society.

17. The following Committees and Subcommittees were discharged, with commendation and thanks for their efforts on behalf of the Society: Fungicides, Special Problems, Fungus Nomenclature, and Nomenclature of Plant Pathogenic Bacteria.

18. The Subcommittee on Utilization of Plant Pathologists and Facilities in National Emergencies is raised to full-committee status and designated as a Special Committee; the Subcommittee on Testing and Results of Newer Fungicides is raised to full-committee status; the Subcommittee on Fungicide Nomenclature is raised to full-committee status.

19. The AIBS is commended for its initiative and activities in connection with the development of the Science Roster and the formulation of plans and policies for effective utilization of plant pathologists and other biological scientists in national emergencies. The Secretary is instructed to inform the AIBS of this action.

CURTIS MAY, *Secretary*